CXCVI. FERMENTABLE SUGAR IN NORMAL URINE.

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INTRODUCTION.

THE problem of true sugar in blood has now received a satisfactory solution. There may be still a little doubt as to whether a few mg. may not be a true sugar other than glucose, but within that limitation the amount can be estimated readily and accurately. It is not so with the presence of true sugar in urine. The urine of all normal persons contains carbohydrate reducing material, but to what extent this consists of glucose has been a matter of controversy. The older work has already been reviewed from time to time [Benedict, Osterberg and Neuwirth, 1918; Folin and Berglund, 1922, 2; Greenwald, Gross and Samet, 1924]. As regards more recent work, there have been no fundamentally new methods of analytical approach to the problem. Observers have still to depend on a yeast fermentation process, or on the formation of osazones.

The danger of contamination by bacteria, in using yeast for the estimation of glucose has been well recognised. Excluding this error, Lund and Wolf [1926] failed to observe CO_2 formation as a result of the incubation of pure yeasts and urine, and Patterson [1926] by incubating ordinary yeast and urine in presence of toluene noted no change in reducing power. Hiller, Linder and Van Slyke [1925] by using larger amounts of yeast shortened the time required for the removal of glucose from aqueous solutions and blood. Eagle [1927] applied the method to urine, and reported the entire absence of fermentable sugar, either fasting or after ordinary meals. His figures have received a correction from Van Slyke and Hawkins [1929], but even so the amount must be under 10 mg./100 cc. urine. On the other hand Greenwald, Gross and Maguire [1927], using Eagle's technique found fermentable sugar after meals but none after glucose in some subjects, and concluded that normal urines contain reducing substances other than glucose that are removed by treatment with yeast. In a series of after-breakfast urines Van Slyke and Hawkins have noted significant amounts of fermentable sugar. Peterson and West [1929] have also observed in 50 normal urines daily excretion of fermentable reducing substances in amounts varying widely up to 400 mg.

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The use of phenylhydrazine has led to similar contradictory evidence, Patterson [1926], Geelmuyden [1915], Höst [1923], have all failed to prepare *d*-phenylglucosazone from normal urines, contradicting the earlier positive claims. More recently however, Hassan [1928], removing interfering substances by charcoal, prepared typical glucosazone crystals from urine 1–2 hours after an ordinary meal in 20–30 % of 700 Egyptian students. After a 12 hours fast the occurrence of glucose was reduced to 7 %. Glucosazone may be valueless as a specific indication of glucose in urine. Quite apart from the possible presence of fructose or mannose, Greenwald, Gross and Maguire [1927] claimed the isolation of *d*-phenylglucosazone from normal urine which contained only non-fermentable carbohydrate reducing material, and drew attention to the possible formation of compounds of this type from sucrose.

A very complete examination of the problem was made by Malmros [1928] who, from evidence of osazone formation combined with yeast fermentation concluded that "even though it has not been possible to produce a chemical-analytically perfectly valid proof, the investigations still indicate that glucose occurs in normal urine."

Other investigators have approached the problem from the physiological standpoint. Thus Folin and Berglund [1922, 2] cited the evidence of blood-sugar studies after the ingestion of large amounts of pure glucose, together with the non-occurrence of any rise in the carbohydrate reducing material in the urine, against the possibility of the occurrence of glucose in normal urine. The experimental work and its value was severely criticised by Benedict and Osterberg [1923], who together with Neuwirth [1918] had put forward a view involving a continuous secretion of glucose into the urine.

Both from the clinical and the laboratory side of medicine during the last decade, there has come an increasing recognition of two types of normal people who consistently show glycosuria. The first, renal glycosuriacs [Goto, 1918; Marsh, 1921], possess a sugar threshold below the normal value and both in the fasting condition and after meals show sugar in the urine. It is recognised too that the threshold for sugar can be found at any level of the blood-sugar [Goto and Kuno, 1921; Gray, 1923; John, 1923], so that individuals will occur who show absence of glucose from fasting urine, but its presence after glucose ingestion or after meals, though the blood-sugar curve may not reach the usual normal renal threshold. The second type have been called "cyclic hyperglycaemics" [Holst, 1925; Faber, 1926] or "lag type people" [MacLean, 1924; Linder, Hiller and Van Slyke, 1924]. In them the blood-sugar rises transiently above the usual value for the renal threshold after glucose ingestion, or after meals, with resulting glycosuria.

In discussing the presence of glucose in normal urine it is usually assumed that these types are not very common, and in experiments on a small series of subjects such types are excluded by direct observation. The real problem of glucose in normal urine lies in determining whether individuals showing no clinical tests for sugar, and presumably possessing glucose tolerance curves

of the "ideal" type [MacLean, 1924; Linder, Hiller and Van Slyke, 1924], actually excrete small amounts of glucose. In view of the contradictory evidence which we have summarised, four possibilities present themselves.

(1) A difference between the fasting condition and after meals.

(2) The occurrence of subjects with thresholds for sugar lower than normal but not low enough to allow the escape of sufficient sugar to be detected clinically.

(3) The occurrence of subjects responding to glucose or carbohydrate ingestion by a hyperglycaemia above the normal threshold, but not showing sufficient glycosuria to be detected clinically.

(4) The urinary sugar reported as fermentable, or as giving *d*-phenylglucosazone, is not glucose.

Experimental.

Analytical methods.

The analytical procedures for urine are as follows:

A. Preliminary dilution. Normal urine is diluted 5 or 10 times. Urines showing positive Benedict sugar tests are diluted 20, 50 or 100 times. Very dilute urines are diluted only 1:1 or 1:2. The dilutions are arranged so that the final titration lies between the values 0·3 and 1·5 cc. of 0·005 *N* thiosulphate solution. It is to be noted that the final dilution is always twice the preliminary dilution.

B. Total "sugar." 5 cc. of the diluted urine, 5 cc. of 0·04 *N* H_2SO_4 are mixed, 0·3 g. Lloyd's reagent added; the mixture shaken and centrifuged. 2 cc. of the supernatant liquid are mixed with 2 cc. of the Shaffer-Hartmann copper solution and used as described for pure glucose under the heading "Standardisation of the copper solution."

C. Non-fermentable "sugar." 1 cc. of a 25 % suspension of washed yeast is centrifuged, the fluid adherent to the centrifuge tube is removed by filter-paper, and to the cells are added 5 cc. of the diluted urine. The cells and liquid are mixed and incubated 8 mins. at 38°, the mixture being agitated two or three times. 5 cc. 0·04 *N* H_2SO_4 are added, mixed and then 0·3 g. Lloyd's reagent, added. After shaking and centrifuging 2 cc. of the supernatant liquid are used for the determination of non-fermentable "sugar" as in total sugar.

D. Blank determination. 5 cc. water are mixed with 5 cc. 0·04 *N* H_2SO_4; 0·3 g. Lloyd's reagent added, and the mixture is shaken and centrifuged. 2 cc. supernatant liquid are used, as in determinations B or C. A blank determination must be carried out with each batch of reductions.

E. Fermentable sugar. The difference B–C properly corrected for the blank represents fermentable sugar.

Recovery of added sugar from urine. Added glucose (100 mg./100 cc.) is recovered either directly or by fermentation from all human urines so far examined. Fructose, however, in concentrations greater than 25 mg./100 cc. is not recovered quantitatively from urine by yeast under our conditions. In lower concentrations the recovery is quantitative.

In presence of a mixture of phenols representing 30 mg./100 cc. phenol the recovery of glucose is similar to that from urine.

Preparation of a modified Shaffer-Hartman micro-reagent[1].

Copper sulphate	5·0 g.
Tartaric acid	7·5 „
Sodium carbonate anhyd.	40·0 „
Potassium iodate	0·7 „
Potassium oxalate	18·4 „
Water to	1000 cc.

[1] The details of this solution and its preparation were kindly supplied to us by Prof. P. A. Shaffer. The notes and criticisms represent the experience of this laboratory after 4 years' use.

The copper sulphate and tartaric acid are dissolved in about 150 cc. of water. The sodium carbonate is dissolved in about 500 cc. of water and the iodate and oxalate in about 150 cc. of water. The solutions are all cooled to 25° and the sulphate-tartaric acid solution added slowly to the carbonate solution with gentle agitation. The temperature is not allowed to rise above 27°. The iodate-oxalate solution is then added and the whole transferred quantitatively to a volumetric flask, and brought to 1000 cc.

A separate solution of 1 % KI.

It is advisable to allow the copper solution to stand 3 weeks before use. This allows a slight amount of precipitate to settle. The solution, once made, does not alter in its oxidising power towards glucose. During the first 3 weeks the oxidising power towards the non-glucose reducing substances in blood alters slightly but after this interval it remains constant. We have used solutions over a year old, without finding any alterations in the values obtained against pure glucose, though the value of the blank increases slightly.

Standardisation of the copper solution. Solutions of pure glucose of 20, 10, 5, 2·5 and 1 mg./100 cc. are prepared.

2 cc. of the copper solution and 2 cc. of the sugar solution are mixed and heated exactly 10 mins. in a rapidly boiling water-bath in a 6″ × ¾″ tube, stoppered with a loose plug of cotton wool. The tube is cooled to 30° and 2 cc. of the KI solution are added. The I_2 is liberated by 2 cc. of N H_2SO_4 with shaking and determined by titration with 0·005 N thiosulphate solution.

We have used 1·0 % starch solution in cold saturated solution of phenol red as indicator. A blank determination with water is also carried out with each batch of reductions. The blank value is subtracted from the sugar value. The difference between duplicate titrations should not be greater than 0·02 cc. of 0·005 N thiosulphate. If desired greater or less amounts of sugar solution can be used with a corresponding increase or decrease in the volume of the reagents.

Sensitivity of the reagent. In water solution the reagents will estimate 0·1 mg./100 cc. of glucose using triplicate determinations.

Stability of reduction products. The solution besides being much more sensitive to glucose than the original Shaffer-Hartman is also superior in the stability of the cuprous compounds, formed in the reduction. The values for glucose over a range of 0·2–10·0 mg. per 100 cc. are the same in an atmosphere of N_2 as under the described ordinary conditions.

Values with blood-filtrates and urines. The method can be used with blood-filtrates prepared by the Folin-Wu method, the Somogyi precipitation with $ZnSO_4$ and NaOH, and the Herbert and Bourne method with 3 % Na_2SO_4 and tungstic acid. 0·3 cc. finger blood precipitated according to Folin-Wu was used for the figures reported in this paper.

Whilst the behaviour of the solution to glucose is constant, we have found variable results when the non-sugar fermentable material of blood is included in the determination [*cf.* Harding and Van Nostrand, 1930]. The cause of this variation we now suspect to be the bicarbonate content of our copper solution. Somogyi [1926] noted the effect of variations in the carbonate-bicarbonate ratio on the glucose values of the Shaffer-Hartman solution and as a result devised a modification containing both carbonate and bicarbonate. A similar variation evidently affects the non-sugar reducing material, as may be shown by passing into the new Shaffer-Hartman solution a stream of CO_2 and determining the values for fermentable and non-fermentable sugar in a Folin-Wu filtrate. Both are lowered, the glucose value to a greater degree than the non-fermentable value.

The values for the non-fermentable sugar in urine have varied very little from one copper solution to another prepared in the usual standard way.

Values with sugars other than glucose. The solution can be used for fructose, galactose and lactose, in water solution, or added to blood-filtrates or urine. Lactates show no reducing values nor iodine absorption values to our reagents.

Use of Lloyd's reagent for removal of interfering substances in urine previous to "sugar" determination. The necessary removal of uric acid, creatinine, *etc.* from urine previously to the application of the modified Shaffer-Hartman reagent may be effected by the original method suggested by Folin and Berglund [1922, 1] of Lloyd's reagent and H_2SO_4. It seems unnecessary to use the modifications of Folin and Svedberg [1926], or Hamilton [1928] under our conditions. The use of Lloyd's reagent, however, does necessitate the determination of a new blank value, in which water previously treated with H_2SO_4 and Lloyd's reagent under the same conditions as urine, replaces water alone.

Removal of fermentable sugar from urine by yeast. We have used Fleischman's yeast washed with water according to the directions of Somogyi [1927]. The stock washed yeast was weighed out when required to make a 25 % suspension by weight with distilled water. We have not found necessary the removal of interfering substances by Lloyd's reagent before the application of the yeast recommended by Eagle [1927].

Van Slyke and Hawkins [1929] have recently stated that yeast applied to urine not only removes fermentable sugar but in addition a variable percentage of the non-fermentable material remaining after treatment with Lloyd's reagent. This results in a correction being necessary in their technique for the determination of fermentable and non-fermentable "sugar" in urine.

We have examined our own technique to see if any such absorption of non-fermentable "sugar" is noticeable. Samples of fasting normal human urine, on which the total "sugar" was first determined, were treated with 3 successive portions of yeast as described in C and the residual non-fermentable sugar determined after each yeast treatment. The results are shown in Table I. The yeast treatments show many times that the urine has gained a little in "sugar" instead of suffering a loss as would be expected were any appreciable amount of non-fermentable yeast-absorbable substance present. The changes in either direction are small, and are of the same magnitude as the thiosulphate titration error. Thus it becomes evident that under our analytical conditions no yeast correction is necessary. Whether the difference between our results and those of Van Slyke and Hawkins lies in the higher dilutions of the urine used by us, or in the use of a copper solution as the reagent for "sugar" determination rather than a ferricyanide reagent, we do not know.

A minor but by no means negligible error can be introduced by yeast in either urines or blood-filtrates by insufficient centrifuging. A very small number of yeast cells, remaining in the supernatant liquid and thus being pipetted into the modified Shaffer-Hartman reagent, causes a marked increase in reduction. In our most accurate work we found it necessary to centrifuge the supernatant liquid a second time.

Table I. *Showing effect of successive treatments with washed yeast on total "sugar" in normal fasting human urine.*

2 cc. of 1:10 diluted urine according to B or C.

Specimen No.	Total "sugar" initial	After yeast treatment 1	2	3	Loss or gain after each yeast treatment 1	2	3
		cc. 0·005 *N* thiosulphate			cc. 0·005 *N* thiosulphate		
1	1·67	1·65	1·62	1·59	−0·02	−0·03	−0·03
2	1·29	1·25	1·27	1·28	−0·04	+0·02	+0·01
3	1·06	1·08	1·06	1·07	+0·02	−0·02	+0·01
4	0·68	0·70	0·72	0·74	+0·02	+0·02	+0·02
5	0·67	0·66	0·64	0·67	−0·01	−0·02	−0·03
6	0·64	0·64	0·64	0·64	±0·00	±0·00	±0·00
7	0·35	0·37	0·35	0·38	+0·02	−0·02	+0·03
8	0·32	0·35	0·35	0·35	+0·03	±0·00	±0·00
	Total change after yeast treatments				+0·02	−0·05	+0·07

Fasting and after glucose.

The subjects represented in Table II were medical students. No food was taken after the meal of the previous evening, the night urine was discarded at 7 a.m., 200 cc. of water were taken, and the student arrived at the laboratory a little before 9 a.m. The subject rested quietly for 15 mins. The 7 to 9 a.m. urine specimen was voided, the initial blood sample taken, and 50 g. glucose in 200 cc. water were given. Finger blood was taken every 15 mins. during the first hour and at the 30 min. interval in the second hour. After 9 a.m. the subjects performed their ordinary laboratory work. An extra 200 cc. of water

was allowed if the 7–9 a.m. specimen had been scanty. The second urine specimen was collected at 11 a.m. All analyses were performed immediately. The blood-sugar curves are shown in Figs. 1 and 2. Table II shows the difference between the total sugar and the non-fermentable "sugar" in cc. 0·005 *N* thiosulphate solution for 2 cc. of urine-filtrate, and the amount of fermentable sugar in the 2-hour sample as glucose. The small amount of thiosulphate—not more than 0·03 cc.—representing the fermentable fraction in the 7–9 a.m. specimen is in the same range of error as two duplicate determinations. The average 0·01 cc. is the same both in the fasting urine and after glucose. Subjects 5 and 9

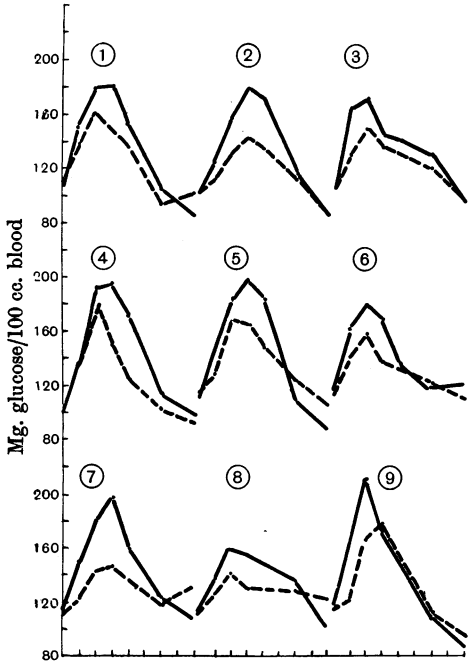

Fig. 1.

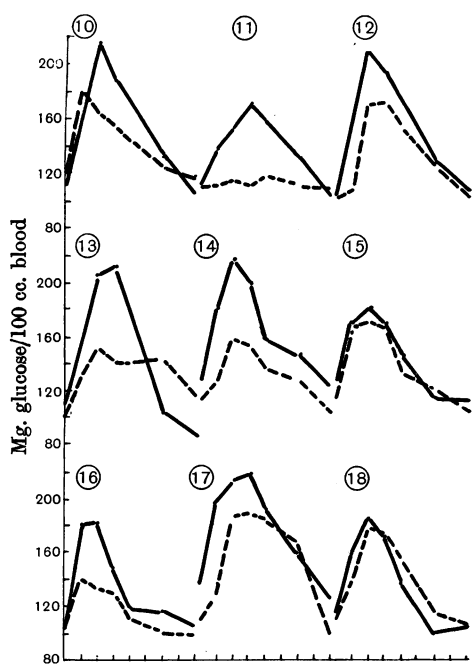

Fig. 2.

Figs. 1 and 2. Showing morning and afternoon tolerance curves after 50 g. glucose.
Morning curves ------- Afternoon curves ————

after glucose show a doubtful result. The amount is so small that the figures can at present have no significance. This does not mean to say that absolutely no glucose escapes into the urine. There may be amounts up to 2 mg./100 cc. present which is the smallest detectable by our method. Peters and Van Slyke [1931] in their recent book, summing up the available evidence, give the actual glucose content of urine as 1 mg./100 cc.

Subject 2 however, has undoubtedly allowed a few mg. of glucose to escape into the urine after glucose ingestion. His renal threshold is a little low, as the maximal blood-sugar observed after glucose ingestion was 142 mg./100 cc. (see Fig. 1). He represents however, only 1 subject out of 18

and it is evident that the occurrence of such variations is not very common. The usual "normal" possesses the usual threshold value for glucose. On the other hand relying on the osazone formation Malmros [1928] is inclined to think that 40 % represents an average rate of occurrence of physiological glycosuria. This is a percentage very much higher than ours. The difference may perhaps be racial, but may be due to our more careful exclusion of any subjects likely to be considered as mild renal glycosuriacs.

Table II.

	7–9 a.m. Fasting		9–11 a.m. 50 g. glucose at 9 a.m.	
No.	0·005 *N* thiosulphate for 2 cc. urine filtrate cc.	Fermentable sugar per 2 hr. mg.	0·005 *N* thiosulphate cc.	Fermentable sugar per 2 hr. mg.
1	−0·005	0·3	−0·01	0·5
2	+0·01	0·3	+0·09	5·1*
3	±0·00	0·0	−0·03	−3·3
4	+0·02	0·8	+0·02	2·8
5	+0·03	2·0	+0·05	2·8
6	+0·03	2·0	+0·02	1·4
7	+0·03	0·0	+0·02	1·2
8	+0·01	0·0	±0·00	0·0
9	+0·03	1·5	+0·04	3·2
10	±0·00	0·0	−0·01	0·0
11	±0·00	0·0	−0·01	0·7
12	±0·00	0·0	−0·02	1·0
13	±0·00	0·0	±0·00	0·0
14	+0·01	1·1	−0·02	−1·5
15	+0·01	0·4	−0·01	−1·8
16	±0·00	0·0	±0·00	0·0
17	−0·02	−0·5	+0·01	0·7
18	+0·01	0·0	+0·03	2·0
Average	+0·01	0·4	+0·01	0·4

* Excluded from average.

In view of the high peaks for blood-sugar shown in the morning curves in Figs. 1 and 2 by some subjects without any glucose in the urine some criticism may perhaps be directed at our analytical methods. The modified Shaffer-Hartman method of sugar analysis we employed sometimes gives slightly higher values for the non-sugar fraction of reducing material present in blood than some of the other reagents. All determinations on blood-sugar reported in this paper, however, were made with one preparation of the reagent whose value to glucose remained constant.

A further series of 15 laboratory workers has yielded us results similar to those of Table II for fasting urine and after glucose ingestion. Some of these are reported in Table I. The occasional occurrence of a few mg. of fermentable sugar in the fasting urine in the morning may perhaps represent a carry-over from the meal of the previous evening, through incomplete removal of the night urine from the bladder, rather than any physiological variation, although we have met with one subject who excreted fermentable sugar in the fasting morning urine after a late meal (midnight), when the urine flow and intake of

water seemed sufficient to ensure thorough emptying of the bladder. This same subject showed minimal fermentable sugar excretion in the 7–9 a.m. specimen if the previous evening's meal was taken 6–7 p.m. In precise work the time relation of the previous evening meal and the fasting specimen cannot be disregarded.

In the remainder of the work reported in this paper we have taken a difference of 0·05 cc. (or over) of 0·005 *N* thiosulphate, between the total "sugar" and the non-fermentable "sugar" to mean the presence of fermentable sugar. Differences less than 0·05 cc. are reported as zero. By this procedure, perhaps, we have failed to observe the excretion of some small quantities of fermentable sugar; on the other hand, we have made certain that those amounts reported as present have a real significance.

Post-prandial.

The urines represented in Table III were collected 2–4 p.m. from a similar group of students. The urines were approximately 2 hours post-prandial. Half the results were obtained in November 1929 and half in October 1930.

Table III. *Fermentable sugar in post-prandial afternoon urine.*

Fermentable sugar mg./100 cc.	Fermentable sugar mg./100 cc.	Fermentable sugar mg./100 cc.	Fermentable sugar mg./100 cc.
7	7	5	4
0	0	19	14
0	5	12	0
0	36	17	0
0	0	16	20
6	0	18	8
0	0	11	6
7	?	0	0
0	6	0	10
21	0	0	0
16	6	0	0
12	13	0	0
0	0	4	8
33	8	10	12

The results, however, are the same. About 50 % of each group show unmistakably fermentable urinary sugar. Our results thus agree in general with those of Van Slyke and Hawkins, Hassan, Peterson and West, and Malmros, that it is not an uncommon finding. The significance of the remaining 50 % who show no fermentable sugar must not be overlooked. The negative results of Folin and Berglund, Eagle, and Greenwald, Gross and Samet could obtain equal support. Post-prandial urines from 8 normal women showed 4 containing fermentable sugar.

Several questions at once arise, the most important of which is the nature of the fermentable sugar. Glucose is not the only reducing sugar removable by yeast. Fructose and mannose are also fermentable and have been shown to be partially removable by yeast under the conditions of Raymond and Blanco [1928]. Fructose in aqueous solution can be distinguished from glucose and mannose by several reactions. The most notable of these is the Seliwanoff

reaction. The diphenylamine reaction has also been used in a quantitative manner by Van Creveld [1927] and Corley [1929]. Neither of these reactions yielded satisfactory results when applied to the urines containing fermentable sugar. We consequently fell back on indirect methods of approach.

Afternoon glycosuria.

In the endeavour to find conditions under which glucose might occur in normal urine the following experiment was performed on 5 laboratory workers. Fasting urine was collected 7–9 a.m. At 9 a.m. 50 g. glucose in water or weak tea were taken followed by ordinary breakfast. Two-hour specimens were collected throughout the day until 7 p.m. Lunch was taken at 1 p.m., and at 5 p.m. 50 g. glucose were again taken. Dinner was taken at 7 p.m. with a night specimen of urine from 7 p.m. till 7 a.m. next morning. In giving glucose with an ordinary breakfast we were influenced by the claims of Benedict and his co-workers [Benedict, Osterberg and Neuwirth, 1918; Benedict and Osterberg, 1923] that glucose taken with meals behaves quite differently from glucose taken in the usual fasting condition. In view of the clear difference just exhibited between fasting and post-prandial urines, it seemed worth while to re-study the question. Greenwald, Gross and Samet [1924] had found also, both in a human subject and in a dog, that glucose given with a high protein diet produced a glycosuria. Our reason for giving 50 g. glucose in the afternoon was the accidental observation, on one of us, that glucose taken at that time produced an unmistakable amount of fermentable urinary sugar, when none was present under conditions of the usual tolerance test. Three of the subjects (V. J. H.; D. L. S.; A. S. C.) had not known of any glycosuria before the test. L. J. H. was known to show sugar in the urine and had a familial history of diabetes, though no symptoms were present. C. E. D. had sometimes shown glycosuria after meals and sometimes was entirely free; there were, however, no symptoms and no history, personal or familial, of diabetes. All had normal fasting blood-sugars.

Table IV. *Afternoon glycosuria.*

	Fermentable sugar every 2 hours in subjects				
Time]	V. J. H. mg.	D. L. S. mg.	A. S. C. mg.	L. J. H. mg.	C. E. D. mg.
7–9 a.m.	0	0	0	0	0
*9–11 „	0	0	0	7	22
11–1 p.m.	0	0	11	7	63
†1–3 „	0	0	7	39	23
3–5 „	0	0	9	7	8
‡5–7 „	132	0	0	174	277
§7–7 a.m.	0	0	0	—	0

* Breakfast + 50 g. glucose. † Lunch *ad lib.* ‡ 50 g. glucose. § Dinner *ad lib.*

The results are shown in Table IV. The two subjects known to show glycosuria excrete recognisable fermentable sugar after the breakfast. One has a few mg. of fermentable sugar during the middle part of the day only. The

remaining two excrete none. The contention of Benedict is evidently not universally applicable. 50 g. of glucose taken with breakfast will not ensure a glycosuria. Nevertheless in view of our own experience with glucose in the afternoon it is possible that his contention may be true of a larger number of people than our few examples would indicate.

Of great interest is the fate of the glucose taken at 5 p.m. In two subjects it evidently had no effect but in the other three it produced a large excretion. Even in subjects L. J. H. and C. E. D. who showed fermentable sugar after breakfast, the excretion was much greater after the afternoon dose. Most interest, however, centres about V. J. H. because (except for the result of a previous similar experiment) it was the first time fermentable sugar had been found in his urine. The zero excretions of fermentable sugar throughout the rest of the day show this subject to be neither a renal glycosuriac, nor a cyclic hyperglycaemic. Previous tests had also shown a normal glucose tolerance curve.

We have termed this phenomenon of glycosuria after a dose of glucose in the afternoon, when it is absent after a fasting dose of glucose, or increased glycosuria in the afternoon when it is present in the morning test "afternoon glycosuria."

For a long time we were uncertain of the real nature of the phenomenon, being unable to control its appearance or disappearance. Thus under ordinary conditions of diet D. L. S. never showed a trace of fermentable urinary sugar, morning or afternoon. V. J. H. showed the phenomenon twice, and then it disappeared on ordinary diets. We consequently carried out a large number of experiments on these subjects in an endeavour to trace some particular influence of the noon meal. These, though they proved failures from our immediate standpoint, led to another possible source of fermentable urinary sugar. Finally by using a new series of subjects, we were able to convince ourselves that afternoon glycosuria is a common occurrence and may represent the concomitant of a regular rhythm in the glucose tolerance.

Table V. *Showing excretion of fermentable sugar 2–4 p.m. and glycosuria 4–6 p.m. after 50 g. glucose at 4 p.m.*

No.	2–4 p.m. mg.	4–6 p.m. mg.	No.	2–4 p.m. mg.	4–6 p.m. mg.
1	12	0	10	2	90
2	19	23	11	0	0
3	12	9	12	12	6
4	0	6	13	6	76
5	0	0	14	0	7
6	26	56	15	0	4
7	0	0	16	6	0
8	0	5	17	6	86
9	4	10	18	6	25

In Table V are shown the fermentable sugar excretions of the 18 subjects of Table II, during the hours 2–4 p.m. and 4–6 p.m. Lunch (*ad lib.*) was to be taken at noon but we cannot be certain that this condition was kept absolutely.

At 4 p.m. they arrived at the laboratory and a glucose tolerance test with 50 g. glucose was carried out, similar to the usual morning one. In view of the claims of Lennox [1927, 1, 2] that the second glucose tolerance test always gives lower blood-sugar levels than the first, half our subjects received the morning test first, and half received the afternoon test first. A fortnight was allowed to elapse between the 2 tests on the same subject. The 2–4 p.m. specimens often showed significant amounts of fermentable sugar. In view of the uncertainty of the time of the midday meal, this may represent post-prandial sugar similar to the subjects of Table V. The significant results are 4–6 p.m. when 13 out of 18 show fermentable sugar. This under the circumstances can only be glucose. This is in sharp contrast with the results in Table II when only 1 out of 18 showed unmistakably a little glucose.

Blood-sugar curves at 4 p.m.

In Figs. 1 and 2 are shown the results of the blood-sugar determinations of the 18 subjects whose urinary fermentable sugars are reported in Tables II and V. There is one outstanding difference between the two sets of curves. In the afternoon series the peak of the curve is always higher than in the corresponding morning curve. In a few of the subjects the difference is so small that it could be counted as an experimental error, except that it is always in the same direction. In others, however, the difference is so marked that the results cannot be doubted. In 14 out of the 18 the difference is 20 mg./100 cc. or over. It seems evident to us that the higher peak of the afternoon curves represents a change in the tolerance of the individual towards sugar, either as a result of the day's activities, or as a sequence to the taking of food. It represents a change towards the condition designated as "cyclic hyperglycaemia" by the Scandinavian clinical workers or the "lag type curve" by the English. Some of the curves fulfil the criteria of "lag type" as defined by MacLean. There thus appears to be a greater chance of exceeding the renal threshold for sugar in the afternoon than under the usual fasting morning conditions of a glucose tolerance test. If the average figures for the peaks of our morning and afternoon curves are compared they are 159 and 191 mg./100 cc. glucose respectively. The average difference is not large, but at the peak of the curve it may be quite sufficient to be the cause of a glycosuria in the afternoon when none is present in the morning. It is to be noted that post-alimentary hypoglycaemia occurs in half the afternoon curves.

Are the results with glucose applicable to the occurrence of fermentable sugar in post-prandial urines? We believe they may be. Increases in blood-sugar are not obtained with glucose only. Bread [Jacobsen, 1913], potatoes [MacLean and De Wesselow, 1921; Rosenthal and Ziegler, 1929], rice [Kageura, 1922; Sakaguchi, Matsuyama and Watanabe, 1922], cooked starch [Foster, 1923], mixed meals [Kjer, 1924], have been used to study carbohydrate tolerance by means of blood-sugar findings. In general they give the same type of blood-sugar curve as glucose. The blood-sugar curve may remain below

the renal threshold for sugar, or may exceed it. Jacobsen [1913], using the Bang method of sugar analysis on finger blood, gave 157 g. of white bread to normal individuals and showed a rise in blood-sugar, sometimes to such an extent above the threshold that clinical glycosuria was evident. The test was given, sometimes fasting and sometimes 1½ hours after a light breakfast. There is thus no reason to exclude glucose as a possible post-prandial urinary fermentable sugar. At any rate it is certain from our experiments that the absence of glucose from the urine after its oral administration under fasting conditions in the morning is no argument for its non-appearance at other times during the day.

Our findings are against the conclusion drawn by many workers that the glucose tolerance curve is a constant for the individual [Hansen, 1923; Neilsen, 1928]. Such a conclusion we believe to be approximately correct for fasting conditions. Our morning tolerance curves show little inclination to rise above the usual threshold value. Under the shifting conditions of daily work and food, however, they vary. Moreover, a great deal of the work on which the idea of the constancy of the glucose tolerance test is based is itself open to criticism owing to the use of venous blood (now well known to give much lower peaks at the height of glucose ingestion [Friedenson, *et al.* 1928]), the collection of too infrequent blood samples to catch the peak blood-sugar value (even our 15 min. samples are barely sufficient), and to the use of relatively inexact clinical methods of detecting the glycosuria instead of methods of delicacy equal to those used in the blood analyses. This last reason has probably been a most potent factor in the lack of recognition of this variation in the glucose tolerance curve. All our urines were negative to Benedict's qualitative sugar reagent. With 30-minute blood samples probably only in 5 out of our 18 cases would there be sufficient difference in the afternoon curves to justify a differentiation from the morning curves. This general agreement, coupled with the negative Benedict test in the urine, would be sufficient to label all curves as normal in type, and the majority as approximately constant for the individual.

It might be objected that our results are brought about by the nature of the preceding meal or diet. Our subjects, however, were on their usual diet, and no restriction was placed on the nature of their noon meal. We obtained from each subject a list of the articles of food making up his lunch on the day of the afternoon test. They were very varied. The utmost that can be said is that the lunch was usually a light one. We have, however, precise experiments on the influence of the noon meal on the afternoon glucose tolerance test.

The nature of the noon meal and its effect on 4 p.m. glucose tolerance curves.

There has been a considerable amount of investigation on the effect of the previous diet on the ordinary glucose tolerance test as shown in blood-sugar curves. The most pronounced effect is that of fat contrasted with carbohydrate. High fat—low carbohydrate diets have been reported by Southwood [1923], Greenwald, Gross and Samet [1924], Sweeney [1927], Stenstrom [1927], Odin

[1927], Kohn, Fries and Felshin [1927], Malmros [1928] and Tolstoi [1929] to decrease the tolerance. Starvation [Bang, 1913; Traugott, 1922; Sevringhous, 1925; Pemberton and Foster, 1920; Du Vigneaud and Karr, 1925; Titiso, 1926; Harding and Van Nostrand, 1929; Hines, Boyd and Leise, 1929] where the body lives mainly at the expense of the fat depôts shows similar blood-sugar curves after glucose ingestion. High carbohydrate diets, conversely, increase the tolerance. The effect of protein is not so clear, Kageura [1922] had reported it to decrease the tolerance but Greenwald, Gross and Samet [1924], on an analysis of his diet, concluded that the effect was due to fat. Heinbecker [1928] found the glucose tolerance curves of the Greenland Eskimo living on a diet high in protein and fat to be normal. Sweeney [1927] found blood-sugar curves after 2 days' protein feeding to medical students intermediate in character between those obtained after a similar period of fat or carbohydrate feeding. On general grounds it might thus be expected that the character of the noon meal would influence the glucose tolerance test at 4 p.m. The only doubts that might exist would be the short time available for the effect of the meal, and whether one meal, however distinctive in character, would be sufficient to offset the influence of a previous ordinary diet. The shortest time in which a characteristic diet has been shown to affect a glucose tolerance test is recorded by Malmros who observed the change produced by a high fat diet for 1 day. We adopted 3 characteristic noon meals[1].

(1) *High fat meal:* 130 g. of 50 % cream made up partly as a cream soup, partly as whipped cream flavoured with saccharine, and partly taken in coffee.

(2) *High carbohydrate meal:* 150 g. carbohydrate in the form of potatoes, sweet potatoes, celery, lettuce, beets, carrots, tomatoes, and bread.

(3) *High protein meal:* 100 g. of protein in the form of roast veal and egg-white.

The subjects were laboratory workers; the noon meal was taken precisely at 12 noon; 2-hour urine specimens were collected commencing at 10 a.m. and the glucose tolerance test (50 g.) was commenced precisely at 4 p.m. An ordinary morning glucose tolerance test was performed on the subjects. A fortnight elapsed between each test. The fermentable urinary sugars are reported in Table VI, and the cutaneous blood-sugar curves on 6 of the subjects in Fig. 3. Venous blood-sugar curves on the remaining 2 subjects showed similar results but are not reported.

The urines show an almost remarkable paucity of positive results exclusive of the 4–6 p.m. period. None of the subjects shows fermentable sugar between meals. None is to be reckoned as a renal glycosuriac. It is not to be expected that a glycosuria should follow the high fat meal. No worker as far as we are aware has observed hyperglycaemia after fat. After protein or amino-acid feeding, however, rises in the blood-sugar have been noted. Cammidge, Forsythe

[1] The authors wish to express their thanks to Miss M. J. Porter and Miss L. Bryant of the Diet Kitchen of the Toronto General Hospital for their help, their unfailing courtesy, and the ingenuity with which they prepared the meals.

and Howard [1921] and Petren [1923] have noted hyperglycaemia after protein feeding. Pollak [1922] and Schatti [1923] found increased blood-sugar after amino-acids. Folin and Berglund, using venous blood for analysis and Lundsgaard [1930, 1, 2], on capillary blood, failed to find any increase in sugar either in man or in dogs after amino-acid or protein feeding. Rapport [1930] has recently reviewed this question from the general standpoint of the interconversion of foodstuffs. Our results, however, show no effect upon the urine in the form of fermentable sugar. It is after the high carbohydrate meal, however, that to us, the paucity of results possesses a possible significance. Only

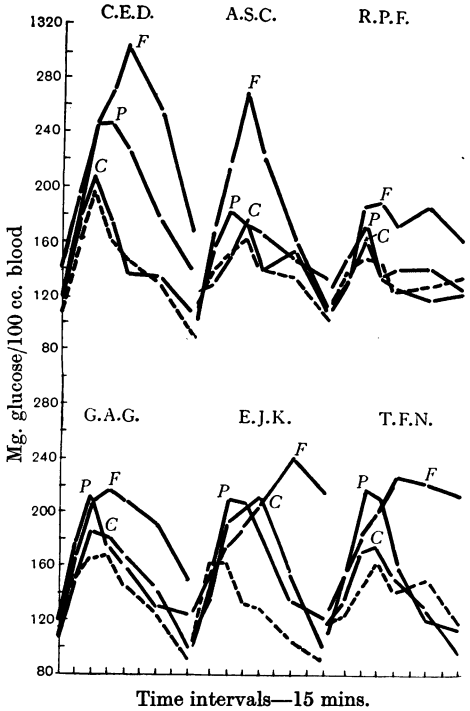

Fig. 3. Showing morning and afternoon tolerance curves after 50 g. glucose, with a variation in the nature of the noon meal.

Morning curves ------- Afternoon curves ————

C = Noon meal of carbohydrate. *P* = Noon meal of protein. *F* = Noon meal of fat.

2 out of 8 show glycosuria in the 12–2 p.m. specimen. In both of these the amount is considerable. In Table III the series of post-prandial urines shows 50 % of fermentable sugars. The amounts however are not large. In Table IV after mixed meals the subjects who show fermentable sugar show small or moderate amounts at hours other than immediately after meals. In Table V also a considerable percentage of subjects show small amounts of fermentable sugar. Our high carbohydrate noon meal contains 150 g. of carbohydrate almost exclusively in the form of starch. There is no cane sugar in the meal except a very small amount left after boiling the beets, carrots and sweet

Table VI. *Fermentable sugar in 2-hour urine specimen.*

Time	C. E. D. Noon meal of C.	C. E. D. Noon meal of P.	C. E. D. Noon meal of F.	A. S. C. Noon meal of C.	A. S. C. Noon meal of P.	A. S. C. Noon meal of F.	R. P. F. Noon meal of C.	R. P. F. Noon meal of P.	R. P. F. Noon meal of F.	G. A. G. Noon meal of C.	G. A. G. Noon meal of P.	G. A. G. Noon meal of F.
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
10 a.m.–12 noon	0	0	0	0	0	0	0	0	0	0	0	0
*12 noon–2 p.m.	141	0	0	0	0	0	0	0	0	0	0	0
2 p.m.–4 p.m.	0	0	0	0	0	0	0	0	0	0	0	0
†4 p.m.–6 p.m.	39	223	1696	12	16	275	0	5	0	8	34	317
Morning glucose tolerance test showed	6 mg.			0 mg.			0 mg.			10 mg.		

Time	E. J. K. Noon meal of C.	E. J. K. Noon meal of P.	E. J. K. Noon meal of F.	T. F. N. Noon meal of C.	T. F. N. Noon meal of P.	T. F. N. Noon meal of F.	V. J. H. Noon meal of C.	V. J. H. Noon meal of P.	V. J. H. Noon meal of F.	D. L. S. Noon meal of C.	D. L. S. Noon meal of P.	D. L. S. Noon meal of F.
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
10 a.m.–12 noon	0	0	0	0	0	0	0	0	0	0	0	0
*12 noon–2 p.m.	168	0	0	0	0	0	0	0	0	0	0	0
2 p.m.–4 p.m.	0	0	0	0	0	0	0	0	0	0	0	0
†4 p.m.–6 p.m.	80	67	479	0	42	367	0	131	738	0	10	0
Morning glucose tolerance test showed	0 mg.			0 mg.			0 mg.			0 mg.		

* Noon meal. † 50 g. glucose. C. = High carbohydrate. P. = High protein. F. = High fat.

potatoes. The fermentable sugar occurring in C. E. D. and E. J. K. after the high carbohydrate lunch must then almost certainly be glucose.

E. J. K. however shows a morning blood-sugar curve allowing the escape of no glucose, and illustrates our contention that the behaviour of the morning glucose tolerance curve is not an exact criterion of the action of carbohydrate at a meal later in the day. In contrast, G. A. G. shows 10 mg. glucose in the morning test and none after the carbohydrate meal. In general, however, the contrast we have drawn between the results shown in Table VI and other post-prandial results gives rise to the suspicion that fermentable sugar other than glucose can occur after meals.

The results of Table VI show afternoon glycosuria in 18 out of 24 experiments. Generally, it appears to be greatest after the fat and least after the carbohydrate noon meal. Two subjects (R. P. F., D. L. S.) show it only after protein, and it seems desirable to emphasise the fact that all the subjects have shown afternoon glycosuria following the noon meal of protein. The blood-sugar curves of Fig. 3 show in all 6 subjects a higher peak in the afternoon than the morning. Of the afternoon curves, those following the carbohydrate noon meal are the lowest, though in E. J. K. and A. S. C. the difference between the peak after carbohydrate and that after protein is very small. The results on the day of the high fat meal are of extreme interest. Three of the tolerance curves are almost diabetic in type. Only in 1 does the sugar value regain its initial value in 2 hours. There is thus not only a high peak but a prolongation in the tolerance curve. The 6 subjects who showed glycosuria gave a positive Benedict sugar test. These same 6 subjects showed marked ketonuria by Rothera's and Gerhardt's tests. It may perhaps be of significance that the 2 subjects (R. P. F. and D. L. S.) who showed no ketonuria also showed no glycosuria.

Malmros [1928] concludes that acidosis is not fundamentally related to the occurrence of glycosuria in non-diabetics but that it may be of importance as a supplementary factor. Our experiments are too few to throw much further light on this problem, but we should like to reserve this aspect of experimental ketonuria for future investigation.

The effect of protein appears intermediate between carbohydrate and fat. An interesting side-issue is the blood-sugar value at 4 p.m. after protein compared with its corresponding value after carbohydrate and fat. The figures are given in Table VII. With one exception, where the subject was continuously nauseated after the meal, the sugar values, both in cutaneous and venous blood were higher after protein than after carbohydrate or fat. In view of the results produced by individual amino-acids on blood-sugar levels in other animals this phenomenon may represent a stimulation of metabolism, rather than a new production of glucose. Lundsgaard [1930, 2] regards the hyperglycaemia from amino-acid feeding as a toxic effect. Also in view of the fact that our method of analysis determines the non-sugar reducing substances as well as true sugar, some uncertainty must attach itself to small differences.

Table VII. *Cutaneous blood-sugar at 4 p.m. after noon meal of protein, carbohydrate or fat.*

Mg. per 100 cc. blood.

Meal	C. E. D.	A. S. C.	R. P. F.	G. A. G.	E. J. K.	T. F. N.	V. J. H.*	D. L. S.*
Protein	140	111†	127	121	127	127	119	114
Carbohydrate	117	122	116	107	111	117	95	110
Fat	120	101	111	109	101	109	110	112

* Venous blood. † Nauseated.

There thus seems to be a good chance of a subject exhibiting glycosuria in response to 50 g. of glucose at 4 p.m. if the noon meal is of mixed character, and, under ordinary variations, the phenomenon will appear independently of the nature of the diet. Variations in individuals, however, are evidently to be expected. The reason for the disappearance of the phenomenon in V. J. H. noted above we now believe to have been an alteration in the glucose tolerance due to repeated experimental administration of glucose which is reported by several observers to improve the tolerance. Eisner [1926] fed glucose daily to rabbits and noted the production of a low type glucose tolerance curve. John [1922] gave 100 g. of glucose to two normal individuals on 5 successive days. The blood-sugar peak on the 1st day was 260 mg./100 cc. glucose; on the 5th day it was 90 mg./100 cc. Sansum, Blatherwick and Bowden [1926], Gibson [1929] and Rabinowitch [1930] have all recently reported the stimulating effect of glucose or carbohydrate feeding on the carbohydrate tolerance of diabetics. It seems very probable that continued administration of glucose may affect also the afternoon glucose tolerance curve. It was a recognition of this possibility that led the authors to select 18 medical students as an entirely new series of subjects for the demonstration of afternoon glycosuria as a common phenomenon.

We can find only a few odd references in the literature bearing on afternoon glycosuria and the corresponding blood-sugar curves. Staub [1921] states that if 20 g. glucose are given 5 hours after a meal, the blood-sugar curve is higher than after a similar dose taken 5 hours later again. Another possibility of rhythm in glucose tolerance may perhaps be found in the work of Kohn, Fries and Felshin [1927], who reported that 2 or 3 days after the change from a high fat diet to a high carbohydrate diet, the glucose tolerance test showed abnormally low blood-sugar curves, and only later gave curves of more normal altitude. This was found in children but has not been noticed by other observers working on adults. The phenomenon may perhaps also have occurred in some of the experiments of Folin and Berglund [1922, 2] for they remark that they "are under the impression that excessive hyperglycaemias are more easily obtained in the evenings than during the early forenoon hours."

Our observations on afternoon glycosuria and its cause apparently bring us into conflict with a widely held view that the carbohydrate tolerance, both of normals and diabetics is lowest in the morning. Naunyn is quoted by Folin and Berglund [1922, 2] as giving this statement as a general expression of clinical opinion on the tolerance of the diabetic in 1906. Peters and Van Slyke [1931] use the morning rise in blood-sugar in the diabetic as illustrating the necessity for the insulin dosage to be highest in the morning. The lowered tolerance of the severe diabetic in the morning may represent one of the differences between that disease and the normal. Such evidence as we have seen, as that of Page [1923], Jonas, Miller and Teller [1925] and Watson [1929], however, by no means inclines us to the view that the normal shows the same lowered morning tolerance. Nor do we wish to be put on record as stating that 4 p.m. blood-sugar tolerance curves are always to be found with a higher peak than those at 9 a.m. in normal individuals. We merely state that such is a common occurrence and leave any discrepancy between our results and those of other observers on normal carbohydrate tolerances to future investigation.

Objection may perhaps be raised to our description of some of the 4 p.m. blood-sugar curves as "lag type." The blood-sugar curves themselves fulfil the criteria of the "lag type" curve. The glycosuria which accompanies our curves, however, is minute compared with "clinical" glycosuria. This difference, however, is only one of degree and we believe may be due to the clinical observations being made on individuals possessing a renal threshold for sugar lower than the usual normal average. Thus a subject possessing a renal sugar threshold of 100–120 mg./100 cc. would show no sugar in the fasting urine with the ordinary clinical tests. After 50 g. glucose, with a usual rise of blood-sugar to 170–180 mg./100 cc. he might show a positive Benedict test. He would then be classed as a renal glycosuriac of the intermediate type described by Graham [1923]. Should his blood-sugar peak rise to 200–220 mg./100 cc. or over it is almost certain that urinary sugar would be found. He would then be classed as a "lag type." Although this may be the explanation of the clinical glycosuria accompanying the "lag type" curve, it does not explain why such

individuals show a "lag type" curve in the morning while our normals only show the high peak in the afternoon.

Urinary fermentable sugar after fruits, honey, orange juice and invert sugar.

In the preceding sections of this paper we have given cogent reasons why glucose may at times be a constituent of normal urine. At the same time doubts have been expressed on the single nature of the urinary fermentable sugar after meals under all conditions. These suspicions were engendered by observations on the authors in their preliminary search for conditions under which afternoon glycosuria might be demonstrable. They took a series of noon meals of varied character. The high fat, protein and carbohydrate, with their negative results, have already been discussed in connection with Table VI. In addition, the effect of variations in the proportions of protein, fat and carbohydrate were studied, using the same food materials. Entirely negative results were obtained. When, however, in high carbohydrate meals a large proportion of the carbohydrate arose from fruits, small amounts of fermentable sugar made their appearance in the urine. The details of some of the meals and the results of some of the experiments are shown in Table VIII. In all, 19 experiments of this nature were performed and fermentable urinary sugar was

Table VIII. *Excretion of fermentable sugar after meals containing large amounts of fruit.*

Expt.	Character of noon meal		Subject	Fermentable sugar 12 noon–2 p.m.	Fermentable sugar 2–4 p.m.
1	High protein and fat	(*a*)	V. J. H.	0	0
			D. L. S.	0	0
2	(*a*) + 60 g. cane sugar		V. J. H.	0	0
			D. L. S.	0	0
3	Mixed	(*b*)	V. J. H.	0	0
			D. L. S.	0	0
4	Mixed	(*c*)	V. J. H.	0	0
	Mixed	(*d*)	D. L. S.	0	0
5	Carbohydrate (fruit salad)	(*e*)	V. J. H.	24	5
			D. L. S.	12	8
6	Carbohydrate (fruits)	(*f*)	V. J. H.	15	0
			D. L. S.	25	0
18	Carbohydrate (fruits)	(*g*)	V. J. H.	18	0
			D. L. S.	8	11
19	Carbohydrate (fruits)	(*h*)	V. J. H.	30	0
			D. L. S.	?	0

(*a*) Oysters, sirloin steak, rolls, butter, coffee, cream, sugar.

(*b*) Lamb, potatoes, carrots, bread, butter, apple pie, tea, cream, sugar.

(*c*) Beef, potatoes, cheese, bread, butter, coffee, apple pie, jelly.

(*d*) Soup, veal, potatoes, corn, bread, butter, cream, coffee.

(*e*) Salad composed of potato, celery, apple, cherries, nuts, mayonnaise; bread, butter, apple pie, cheese, coffee, sugar.

(*f*) Grapefruit, prunes, apricot, peaches, bread, butter, apple pie, coffee, cream, sugar (50 g.).

(*g*) 150 g. potato, 80 g. carrot, 70 g. sweet potato, 120 g. orange (edible portion), 100 g. banana (edible portion), 200 g. orange juice, cream, coffee.

(*h*) Largely orange and banana, small amount potato and carrot, 200 g. orange juice.

clearly demonstrated only in 4, *viz.* when the meal contained a large amount of fruit. Since neither author is in the habit of showing demonstrable urinary fermentable sugar after ordinary meals, the positive results after fruit, in both authors, and occurring under the same conditions, become thus more remarkable and point to the occurrence of fermentable sugar in urine other than glucose.

We extended our observations to two other subjects; one of whom, judging by the results in Table VI, possesses as efficient a mechanism as D. L. S. for the retention of glucose. To these subjects we gave 500 cc. of orange juice at 9 a.m. under the conditions of a morning glucose tolerance test. The juice was made from 12 Florida oranges, extracted the previous afternoon, and kept overnight in a refrigerator. It represents 50 g. edible carbohydrate. The results are shown in Table IX. Both showed fermentable urinary sugar. A repetition of the experiment gave the same result. Honey, and 50 g. of invert sugar prepared in the laboratory by the action of 0·5 *N* HCl on cane sugar, also gave fermentable urinary sugar. Addition to the orange juice of (*a*) glucose, (*b*) cane sugar, (*c*) invert sugar did not increase the urinary fermentable sugar. 50 g. cane sugar gave negative results. In view of this result we observed the action of glucose which had been allowed to stand overnight with 0·5 *N* HCl, in case the action of the acid might produce small amounts of fermentable material which escaping into the urine would be reckoned as glucose, though it is more usual to suppose the stability of sugars under such conditions. The result of the experiment was negative.

Table IX. *Excretion of fermentable sugar after orange juice, honey and invert sugar.*

Expt.	Ingestion at 9 a.m. of	Subject	Fermentable sugar 7 a.m.–9 a.m.	9 a.m.–11 a.m.	11 a.m.–1 p.m.
			mg.	mg.	mg.
20	500 cc. orange juice	R. P. F.	0	7	0
		C. E. D.	0	7	0
21	500 cc. orange juice + 40 g. cane sugar	R. P. F.	0	12	0
		C. E. D.	0	12	0
22	90 g. cane sugar in 500 cc. water	R. P. F.	0	0	0
		C. E. D.	0	0	0
23	500 cc. orange juice	R. P. F.	—	5	0
		C. E. D.	0	15	0
24	500 cc. orange juice + 40 g. glucose	R. P. F.	0	13	0
		C. E. D.	0	7	0
25	500 cc. orange juice + 40 g. partly hydrolysed cane sugar	R. P. F.	0	18	6
		C. E. D.	0	28	0
26	500 cc. orange juice + 40 g. invert sugar	R. P. F.	0	0	0
		C. E. D.	0	15	0
27	90 g. invert sugar in 500 cc. water (50 cc. *N*/2 HCl)	R. P. F.	0	8	0
		C. E. D.	0	10	0
28	120 g. honey in 500 cc. water	R. P. F.	0	5	0
		C. E. D.	0	15	5
29	50 g. glucose + 50 cc. *N*/2 HCl in 500 cc. water	R. P. F.	0	0	0
		C. E. D.	0	0	0

Such a series of experiments could point only to the presence of free fructose, some decomposition product by acid, or a new substance removable by yeast and formed as a metabolic product from fructose. We feel our experiments may offer a partial explanation of some of the results in the literature on post-prandial sugar. The use of jams, preserves, and marmalade, where acid fruits are boiled with cane sugar, might offer another source of urinary fermentable sugar.

Urinary fermentable sugar after fructose.

On six subjects we examined the action of 50 or 25 g. of fructose both at 9 a.m. under the usual fasting conditions, and at 4 p.m. The fructose used was Schuchart's "puriss. cryst." ($[\alpha]_D^{20} - 92{\cdot}5°$). The urine findings are given in Table X and the capillary blood-sugars in Fig. 4.

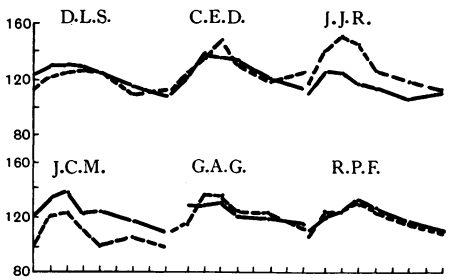

Fig. 4. Showing morning and afternoon tolerance curves after 50 or 25 g. of fructose.
Morning curves -------- Afternoon curves ————

All subjects showed fermentable urinary sugar both in the morning and afternoon. The amounts are small, and are of the same magnitude as those shown after fruits, *etc.* in the preceding section. Moreover in the morning experiments there is seen an excretion lasting 2–4 hours after the administration of the sugar in three of the six subjects. A similar continued excretion is noticed in some of the experiments after fruit, *etc.* and in some of the post-prandial sugars. We have referred to those in Table IV. Some of those in Table V may also represent a similar continued excretion. Recently Corley [1929] has shown the presence of small amounts of urinary fructose after fructose feeding in the rabbit. The presence of fructose in the blood-stream after fructose feeding has been shown in man by Folin and Berglund [1922, 2] using the Seliwanoff test, and by Corley in the rabbit using the modified Van Creveld method. There thus seems no reason to refuse to label the urinary fermentable sugar in our experiments after the feeding of fructose, fruits, honey or invert sugar, as fructose, unless some unidentified metabolic product behaves in a similar way. That fructose differs from glucose in its metabolism is well known. It possesses its own rate of absorption [Cori and Cori, 1925]. Its high ability to form glycogen [Cori, 1926], its poor utilisation in the muscles [Mann and Bollman,

1930] and the accompanying formation of lactic acid after oral administration [Campbell and Maltby, 1928] all mark it off from glucose.

Table X. *Urinary fermentable sugar in six subjects after oral administration of fructose at 9 a.m. and at 4 p.m.*

Subject	Fructose ingested	Fermentable sugar in 2 hr. urine: Fructose at 9 a.m. 9–11	Fructose at 9 a.m. 11–1	Fructose at 4 p.m. 4–6
	g.	mg.	mg.	mg.
D. L. S.	50	7	4	8
C. E. D.	50	7	0	8
J. J. R.	25	21	0	13
J. C. M.	25	14	0	13
G. A. G.	50	19	10	13
R. P. F.	50	8	0	9

The differentiation from glucose is also to be noticed in the shape of the blood-sugar curves after ingestion. The total blood-sugar is only elevated a little in comparison with the rises noted after glucose. The morning curves shown in Fig. 4 confirm many observations in the literature. The afternoon curves are almost exactly similar to the morning curves. There is no evidence of any afternoon fructosuria either from the urinary findings, or from any increased peak or height in the blood-sugar curves. This again differentiates its metabolism from glucose. At present we do not wish to offer any explanation, either of the phenomenon of afternoon glycosuria or of our failure to observe a similar phenomenon after fructose.

General conclusions.

1. Within the limits of the analytical methods fermentable sugar is absent from normal fasting urine. The possible amounts of fermentable sugar must be under 5 mg./100 cc. and are more likely to be less than 2–3 mg./100 cc. We have excluded known renal glycosuriacs from our observations.

2. After glucose ingestion in the morning under fasting conditions no increased amount of fermentable sugar can be detected in the urine. A survey of a large number of individuals will, however, undoubtedly reveal some showing glycosuria, though too small to be detected by the usual clinical reagents. The percentage in which such individuals occur in a series will influence the experimenter to declare the presence or absence of glucose in normal urine after glucose ingestion. Three such examples occur in our experiments. In one there appears to be a slightly lowered renal threshold.

3. 50 % of medical students show small amounts of fermentable sugar in the post-prandial afternoon urine.

4. Two subjects who showed no fermentable urinary sugar, fasting, after glucose in the morning, or after ordinary mixed meals, showed fermentable sugar after large amounts of fruit.

5. Fermentable urinary sugar was also shown to occur in two other subjects after orange juice, honey and invert sugar, taken fasting.

6. 50 or 25 g. of pure fructose given as a fructose tolerance test in the morning or in the afternoon at 4 p.m. give rise to urinary fermentable sugar.

7. 50 g. glucose at 4 p.m. give rise to glycosuria in individuals showing none under the fasting conditions of the morning test. This phenomenon of afternoon glycosuria is of frequent occurrence. It is least likely to occur if the noon meal is high in carbohydrate. It has always occurred if the noon meal is high in protein. A high fat noon meal is also very likely to cause afternoon glycosuria.

8. The blood-sugar curves at 4 p.m. taken under ordinary conditions of diet all possess a higher peak than the corresponding fasting morning tolerance curves. The peak often rises above the usual renal threshold for sugar. This accounts for afternoon glycosuria. Some of the blood-sugar curves resemble those of the MacLean "lag type."

9. The nature of the noon meal affects the blood-sugar curve after 50 g. glucose at 4 p.m. A high carbohydrate meal gives rise to the lowest curve. A high fat meal gives rise to a high peak curve, sometimes remaining high at the end of 2 hours. Ketosis was observed in six out of eight experiments after our high fat noon meal. A high protein noon meal gives a high peak blood-sugar curve. All tolerance curves at 4 p.m. are higher than the morning tolerance curve in the same individual.

10. We have found no phenomenon corresponding to afternoon glycosuria in the urine, or in the shape of the blood-sugar curves, after the oral administration of fructose. This may serve as additional evidence differentiating the metabolism of glucose from that of fructose.

11. The non-occurrence of glycosuria after the usual morning glucose tolerance test cannot be taken as a criterion of its continued non-occurrence throughout the day. After a noon meal where the carbohydrate consisted almost entirely of starch a subject showed glycosuria, although a morning tolerance test with glucose was entirely negative. Another subject showed a few mg. of glucose in the urine after the morning test, but none after the carbohydrate meal.

12. The occurrence of fermentable sugar in normal urine is a variable phenomenon depending on a number of complex factors.

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