CCXVIII. FERMENTABLE SUGAR IN FASTING URINE.

BY VICTOR JOHN HARDING AND DAVID LONGLAND SELBY.

*From the Department of Pathological Chemistry, University of Toronto,
Toronto, Canada.*

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THERE seems no doubt that the post-prandial urine of normal men and women often contains a reducing substance or substances removable by yeast. This "fermentable sugar" has been found after breakfast by Van Slyke and Hawkins [1929], at irregular intervals during the day both by Greenwald *et al.* [1927] and by Harding and Selby [1931], and in 24 hour urines by West and Peterson [1932]. Its occurrence varies with the nature of the meal (Harding and Selby) or the general diet [West, Lange and Peterson, 1932] and with the individual (Harding and Selby). Whilst all the methods for its determination have in common the use of yeast, different reagents have been used for the determination of the reducing power, thus minimising the possibility that the findings are due to some extraneous accident of technique. The fraction removable by yeast almost certainly contains glucose. The fraction is found in the carbohydrates of the urine. Harding and Selby provided physiological evidence for the entry of glucose from the blood, and West and Steiner [1932] state that the rate of fermentation is almost exactly paralleled by the same amount of glucose under the same conditions. On the other hand Greenwald *et al.* believed it to be non-glucose in character, and Van Slyke and Hawkins have clearly shown the simultaneous occurrence of small amounts of irregularly excreted non-glucose substance removable by yeast. Harding and Selby also showed the possible occurrence of fructose.

The previous paper of Harding and Selby reported the practical absence of a substance removable by yeast from fasting urines and at many other times during the 24 hours. We did not deny the total absence of glucose, but recognising the limitations of the method of analysis, regarded such amounts of that sugar as might be present in these urines as minimum. Certainly there was no difficulty in differentiating between these possible minimum amounts and the comparatively large excretion of post-prandial substances removable by yeast. This irregularity of excretion, affecting both the time of day and the individual, enabled the authors to harmonise their own results with the previous contradictory findings reported in the literature.

In a recent series of papers West and his colleagues [West and Peterson, 1932; West, Lange and Peterson, 1932; West and Steiner, 1932] claim that fermentable sugar occurs in all urines. This they identify as glucose.

The crucial point of difference between ourselves and West *et al.* lies in the possible amount of fermentable sugar in fasting urine. West and Peterson ascribe our lack of ability to find fermentable sugar in fasting urines to the operation of a number of causes which, acting together, reduce the effective amount of this substance to such an extent that the titration difference between the total sugar and non-fermentable sugar becomes indistinguishable from the

titration error. They calculate that the presence of 6 mg./100 cc. fermentable sugar in urine at a dilution 1 : 10 would only mean a titration difference of 0.08 cc. 0.005 *N* thiosulphate under our conditions, and a difference of 0.04 cc. at dilution 1 : 20. West, Lange and Peterson go so far as to remark "that the method used by these workers is incapable of estimating with sufficient accuracy the fermentable sugar of starvation urines."

In our previous paper we drew the conclusion that "the possible amounts of fermentable sugar in normal fasting urine must be under 5 mg./100 cc. and are more likely to be less than 2-3 mg./100 cc." The results on which this conclusion is based are to be seen in Tables I and II of the previous paper. In Table I, where the dilution is constant at 1 : 10, only 1 urine out of 8 shows a titration difference of 0.04 cc. Evidently in this series of urines, the amount of fermentable sugar is 3 mg./100 cc. or less, and such a conclusion neglects the fact that some of the urines gained slightly in reducing power, rather than lost. In Table II the dilutions are varied, but only 4 out of 18 urines show fermentable sugar equal to 0.03 cc. 12 out of the 18 show titration differences of +0.01 or 0.00 cc. The average result lies within our experimental error, and we consider our conclusion justified. Our conclusions were supported also by an unpublished series of observations on 8 normal fasting urines of which we now give the details of 4. The methods were those of our previous paper.

Extreme care was taken in this series. All apparatus was recalibrated and especially cleaned before use. The yeast was most thoroughly washed and after use on the diluted urine was removed by a double centrifuging. Two samples from each urine specimen were analysed separately, with duplicate titrations on each. The extreme range of all 4 titrations did not exceed 0.02 cc. 0.005 *N* thiosulphate. The average value thus only varies from the extreme by ± 0.01 . Table I shows the results of 2 urines diluted 1 : 10 and 2 diluted 1 : 20. The amount of sugar removable by yeast is represented by differences of ± 0.01 cc.

Table I.

TABLE I.					
Subject	Specimen	Final dilution	Total sugar	Non-fermentable sugar	Fermentable sugar
			cc. 0.005 <i>N</i> thiosulphate per 2.0 cc. filtrate		
I	1	1 : 10	1.02	1.03	
			1.02	1.02	
	2		1.01	1.03	
			1.02	1.01	
	Average	1.02	1.02	0.00	
II	1	1 : 10	0.83	0.84	
			0.83	0.83	
	2		0.82	0.84	
			0.83	0.84	
	Average	0.83	0.84	- 0.01	
III	1	1 : 20	0.57	0.55	
			0.57	0.54	
	2		0.55	0.55	
			0.56	0.55	
	Average	0.56	0.55	+ 0.01	
IV	1	1 : 20	0.66	0.64	
			0.64	0.65	
	2		0.65	0.65	
			0.64	0.64	
	Average	0.65	0.65	0.00	

It would seem a justifiable conclusion that fermentable sugar, or sugar removable by yeast, was present in these urines only in minimum amounts. Had we been dealing with aqueous solutions of glucose we should have detected with certainty 1–2 mg./100 cc. With urines the sensitivity of our reagents appeared somewhat less, and we set the limits of detection at 2–3 mg./100 cc. Our reagents and methods were of sufficient delicacy to detect glucose in the concentrations stated by West *et al.*

So far neither West *et al.* nor ourselves have confirmed the presence in urine of the non-glucose reducing substance removable by yeast of Van Slyke and Hawkins. These latter obtained evidence for its existence in urines diluted only 1 : 1, when acted on by an equal volume of washed centrifuged yeast cells. There was possibly some indication of its existence in the urine No. 1 in Table I of our previous paper, where 3 successive treatments with yeast removed equivalents of 0.02, 0.03 and 0.03 cc. 0.005 *N* thiosulphate. The amount was very small, so small that we felt no correction was required in the majority of urines. Nevertheless we minimised the possible interference of this substance by using a highly diluted urine. At the same time in our study of post-prandial urines we refused to consider as true fermentable sugar any difference less than 0.05 cc. 0.005 *N* thiosulphate. The use of urines of only moderate dilution, and the interpretation of very small diminutions in reducing power after the action of yeast as true fermentable sugar, were open to the criticism that we were dealing with the Van Slyke-Hawkins or a similar substance, which when present in very small amounts became completely removable by a single yeast action.

Although as a result of West's criticism we have felt ourselves under the necessity of defending and amplifying our previous work, nevertheless it is a matter of surprise that there is any serious matter for disagreement. Both laboratories use the same general method of analysis, the yeast preparations and their use are the same, and the copper reagents differ only in a few details. Both methods quantitatively recover added glucose, either as a direct addition to the total sugar value, or as extra fermentable sugar. If the fermentable sugar found by West *et al.* in fasting human urine consists entirely of glucose, the two sets of observations should be in substantial, if not complete, agreement. True, West uses $\text{HgSO}_4\text{--BaCO}_3$ in his preliminary treatment of the urine and we have used $\text{H}_2\text{SO}_4\text{--Lloyd's reagent}$. This however should affect only the level of the total sugar and non-fermentable sugar determinations and not their difference, if glucose is the sole urinary fermentable sugar. The present paper contains the results of some observations directed towards discovering if possible the cause of the discrepancies.

GENERAL METHODS.

The general method of analysis is the same as in our previous paper. After dilution the urine is treated with a suitable reagent to remove interfering substances. The total "sugar" is determined on 2 cc. filtrate. Another portion of the diluted urine (10–15 cc.) is treated with 0.25 g. wet-weight washed centrifuged yeast for 8 minutes at 37° with constant stirring. The yeast is removed by centrifuge. The non-fermentable "sugar" is determined on 2 cc. of this centrifugate after clearing. All thiosulphate titrations were performed in triplicate. Two copper reagents have been used. The first is that described by Harding and Selby [1931] and the second is that described by Harding and Downs [1933]. The chief difference between them is the carbonate-bicarbonate ratio. We shall refer to these reagents as 1 and 2.

All urine-filtrates were adjusted to p_{H} 6.5 previous to "sugar" determinations.

Influence of ammonia on "sugar" determinations.

Harding and Downs [1933] showed that very small concentrations of ammonium salts decreased the sensitivity of their reagent to sugars in aqueous solution. The removal of ammonia could readily be effected by the addition of KH_2PO_4 followed by solid MgO . This gave a filtrate containing the original amount of sugar. The presence of ammonium salts in urines may thus cause a certain amount of error in sugar determinations, unless the preliminary "clearing" at the same time removes the ammonia. In normal urines the amount is not large, and the error, in view of the uncertainty of interpretation to be attached to total sugar determinations, is probably not serious. The use of $\text{HgSO}_4\text{--BaCO}_3$ as recommended by West gives a filtrate free from NH_3 . Lloyd's reagent leaves some NH_3 , but this clearing agent can be preceded or followed by the $\text{KH}_2\text{PO}_4\text{--MgO}$ treatment.

However great or small may be this possible source of error on the determination of total sugar, within the limits of its normal variation, the presence of NH_3 has no effect on the yeast action. There thus appears to be no reason to suspect an inaccuracy of our previous determinations of fermentable sugar from this cause.

A fasting 2 hour urine sample was diluted to 150 cc. To 120 cc. of the diluted urine were added 1.2 g. KH_2PO_4 and 2.4 g. solid light calcined MgO . After shaking 15 minutes the mixture was filtered and the filtrate found NH_3 -free by Nessler's reagent. The filtrate was brought to p_{H} 6.5 by adding 1 or 2 drops of 20 % H_2SO_4 . 100 cc. of 0.04N H_2SO_4 were added to 100 cc. urine-filtrate and 6 g. Lloyd's reagent. After shaking a few minutes, the treated urine was filtered. A determination of total sugar and non-fermentable sugar after yeast action was made. To 50 cc. of the cleared diluted urine were added 50 mg. $(\text{NH}_4)_2\text{SO}_4$. This represents approximately 150 mg. NH_3 per 100 cc. original urine. Total sugar and non-fermentable sugar after yeast action were again determined. The added NH_3 was removed from the remainder of the liquid by KH_2PO_4 and MgO . Total sugar and non-fermentable sugar after yeast action were again determined.

The sugar estimations were made by both copper reagents. The results, shown in Table II, demonstrate the very slight effect of the added ammonium salt when the estimations were made by reagent 1 and the marked effect on reagent 2. The effect, however, is the same on both total sugar and non-fermentable sugar determinations. The yeast action is evidently unaffected.

Table II. *Showing the action of added NH_3 on total sugar and non-fermentable sugar determinations and the accuracy of fermentable sugar determinations in presence of ammonium salts.*

Procedure on urine	"Sugar" as cc. 0.005N thiosulphate per 2 cc. filtrate					
	Reagent 1			Reagent 2		
	T.S.	N.F.S.	F.S.	T.S.	N.F.S.	F.S.
$\text{KH}_2\text{PO}_4\text{--MgO}$ and $\text{H}_2\text{SO}_4\text{--Lloyd's reagent}$	2.82	2.44	0.38	2.07	1.77	0.30
As above + added $(\text{NH}_4)_2\text{SO}_4$	2.80	2.42	0.38	1.75	1.45	0.30
Added $(\text{NH}_4)_2\text{SO}_4$ removed by $\text{KH}_2\text{PO}_4\text{--MgO}$	2.82	2.44	0.38	2.07	1.76	0.31

T.S. = Total sugar.

N.F.S. = Non-fermentable sugar.

F.S. = Fermentable sugar.

The use of KH_2PO_4 -MgO supplies another method for "clearing" urines previous to sugar determinations. This method removes very little reducing material, giving high "sugar" values when used alone. Such urines are Ca-free and contain only a small amount of phosphate.

Effect of variations in methods of clearing of urine and different copper reagents.

If the directions of West and Peterson for the use of HgSO_4 - BaCO_3 are followed and the interfering substances of urine removed by this reagent, there is no difficulty in finding fermentable or yeast-removable sugar in every fasting urine we have examined, at dilutions varying from 1:5 to 1:15 and using 2 cc. filtrate for the titrations. We have found it convenient to express our results in mg. glucose per 2 hours, but assuming a regular excretion throughout the day the amount would vary from 36 to 60 mg. The concentration varies from 4 to 15 mg./100 cc. The yeast was used on diluted urine and previous to the HgSO_4 - BaCO_3 treatment.

If the diluted urine is treated with KH_2PO_4 -MgO followed by H_2SO_4 -Lloyd's reagent, following directions similar to those given in the previous section, and the yeast action is allowed to take place just previous to the treatment with H_2SO_4 -Lloyd's reagent, a series of figures is obtained for fermentable sugar similar to those obtained by the use of HgSO_4 - BaCO_3 . Both results are independent of the copper reagent.

If the treatment or "clearing" of the urine is solely by H_2SO_4 -Lloyd's reagent, two sets of results are obtained for fermentable sugar. The determination of total and non-fermentable sugar by reagent 1 (the reagent used in our previous paper) gives figures for fermentable sugar approximately one-half of those given by reagent 2, or by either reagent after the use of HgSO_4 - BaCO_3 etc. The fermentable sugar is now 1 to 3 mg. per 2 hours, reckoned as glucose, and its concentration varies from 2 to 6 mg./100 cc.

All these results are shown in Table III. The values for fermentable sugar are obtained by subtraction of the non-fermentable sugar from the total sugar values in mg./2 hours and not by calculation from the difference as given in cc. 0.005 *N* thiosulphate. This causes occasional slight discrepancies between

Table III. *Showing the effect of different clearing reagents on the estimation of total (T.S.), non-fermentable (N.F.S.) and fermentable (F.S.) sugar.*

Figures represent mg./2 hours as glucose. Bracketed figures are cc. 0.005 *N* thiosulphate for 2 cc. urine filtrate.

Subject	Vol./ 2 hours cc.	Approx. final dilution	H_2SO_4 -Lloyd's reagent			KH_2PO_4 -MgO and H_2SO_4 -Lloyd's reagent			HgSO_4 - BaCO_3			Copper reagent
			T.S.	N.F.S.	F.S.	T.S.	N.F.S.	F.S.	T.S.	N.F.S.	F.S.	
A.R.A.	34	1:15	36	34	2 (0.06)	40	35	5 (0.13)	24	20	4 (0.11)	1
			28	24	4 (0.15)	28	23	5 (0.15)	21	16	5 (0.15)	2
T.F.N.	94	1:5	49	47	2 (0.05)	51	46	5 (0.15)	30	25	5 (0.14)	1
			32	28	4 (0.13)	35	30	5 (0.16)	25	20	5 (0.17)	2
D.L.S.	55	1:10	50	48	2 (0.04)	50	45	5 (0.12)	27	24	3 (0.09)	1
			31	29	2 (0.09)	33	30	3 (0.11)	23	19	4 (0.12)	2
G.A.G.	56	1:10	54	53	1 (0.03)	52	48	4 (0.13)	30	25	5 (0.14)	1
			37	33	4 (0.12)	37	33	4 (0.12)	26	21	5 (0.12)	2
S.J.	112	1:5	50	47	3 (0.07)	48	43	5 (0.14)	28	24	4 (0.10)	1
			32	28	4 (0.13)	33	28	5 (0.15)	28	24	4 (0.17)	2
C.E.D.	57	1:10	43	41	2 (0.06)	48	42	6 (0.18)	25	21	4 (0.10)	1
			27	22	5 (0.16)	31	26	5 (0.17)	20	16	4 (0.15)	2
C.E.D.	50	1:10	42	40	2 (0.07)	46	40	6 (0.17)	23	19	4 (0.10)	1
			30	26	4 (0.14)	33	28	5 (0.18)	21	17	4 (0.15)	2

the values as shown in the two columns, as we have approximated the values for total and non-fermentable sugar to the nearest mg.

Two questions at once arise. Why does copper reagent 1 compared with reagent 2, and used after H_2SO_4 -Lloyd's reagent, estimate only one-half the fermentable sugar, and why do the results not agree with those of Tables I and II of our previous paper and Table I of the present paper? To the first question it would seem a reasonable answer that reagent 2 determines some non-glucose substance removable by yeast which is estimated either partially or not at all by reagent 1. If so, this substance might have been altered by the previous treatment of the urine by KH_2PO_4 - MgO , for the fermentable sugar is now the same by both reagents. Treatment of urine with HgSO_4 - BaCO_3 apparently also alters its value to the same degree, unless the agreement is entirely fortuitous. It must be remembered that the yeast action in our experiments has taken place before the treatment with H_2SO_4 -Lloyd's reagent or HgSO_4 - BaCO_3 , so that any change should be shown as an apparent increase in total sugar. Of the two copper reagents No. 2 was designed to have a very much smaller oxidising power on some nitrogenous reducing substances, and HgSO_4 - BaCO_3 was designed by West to remove a number of such possible substances. Changes of this character overshadow to such an extent any possible alterations in the reducing power of a small amount of non-glucose substance removable by yeast that no direct indication of the existence of this latter can be obtained from our data. The facts however are inconsistent with glucose being the sole fermentable or yeast-removable substance in urine. The second question is discussed later.

Recovery of added glucose from urine.

In view of the inconsistencies just reported we feel it necessary to give the actual recovery figures of small amounts of glucose from urine in which the clearing agent and the method of using the yeast are varied. Eagle [1927] had advised the use of yeast after H_2SO_4 -Lloyd's reagent. Usually we have used yeast before the clearing agent. We determined added glucose when approximately 2.5 mg. glucose were added to a 2 hour fasting urine specimen and the "clearing" was varied as follows:

- I. urine diluted and "cleared" by H_2SO_4 -Lloyd's;
- II. urine diluted and "cleared" by KH_2PO_4 - MgO ;
- III. urine diluted and "cleared" by KH_2PO_4 - MgO followed by H_2SO_4 -Lloyd's reagent;
- IV. urine diluted and "cleared" by HgSO_4 - BaCO_3 .

Table IV. *Showing recovery of small amounts of glucose added to normal fasting urine. The glucose is recovered as extra fermentable sugar.*

Method	Yeast used previous to "clearing"				Yeast used after "clearing"			
	Glucose added 2.6 mg.				Glucose added 2.7 mg.			
	Fermentable sugar present in urine Reagent		Extra fermentable sugar Reagent		Fermentable sugar present in urine Reagent		Extra fermentable sugar Reagent	
	1	2	1	2	1	2	1	2
I	4.4	3.0	2.8	3.1	3.2	4.2	2.9	2.6
II	3.6	2.6	3.4	2.8	5.5	3.8	2.8	2.8
III	3.8	3.8	2.9	2.8	3.4	4.9	3.4	2.2
IV	3.0	3.7	2.5	2.8	3.4	4.7	3.1	2.4

The preliminary dilution was approximately 1 : 3. The yeast was used on the preliminary diluted urine, before the clearing agent in one series of experiments and after the clearing agent in a second series. The final dilution was 1 : 6. The reducing power was determined by both copper reagents. All methods of clearing and variations in the use of yeast gave satisfactory recovery figures (Table IV).

Effect of dilution preliminary to clearing.

We have stated that to minimise in our previous experiments the effect of any possible non-glucose substances removable by yeast on our determinations of fermentable sugar in normal urine, we used a high dilution. Apart from the limits of our ability to estimate glucose, to what extent did the high preliminary dilution contribute to our previous negative findings?

A. A 2 hour specimen of normal fasting urine was diluted to 150 cc. (dilution approximately 1 : 3). On a portion of this were determined total and non-fermentable sugar, using H_2SO_4 -Lloyd's reagent and allowing the yeast to act previous to clearing. The final dilution, at which the sugar determinations were made, was approximately 1 : 6. Except for a more concentrated urine, the technique follows exactly that of our previous paper.

A/3. Portions of the final 1 : 6 dilution urines from A were diluted with water to a dilution 1 : 18.

B. 50 cc. of the preliminary diluted (1 : 3) urine from A were diluted to 150 cc., making a new preliminary dilution of 1 : 9. On this were determined total sugar after H_2SO_4 -Lloyd's reagent and non-fermentable sugar after yeast and H_2SO_4 -Lloyd's reagent. The final dilution was 1 : 18. Copper reagents 1 and 2 were used in the three sets of determinations.

A comparison of the results from A, A/3 and B should show the same amount of fermentable sugar in mg./2 hours if glucose is the sole fermentable sugar in urine. Expressed in cc. 0.005 *N* thiosulphate per 2 cc. final dilution of urine, the fermentable sugar of both A/3 and B should be 1/3 the value of A, if we are dealing only with glucose. If, however, substances other than glucose constitute part of this fraction, the values for fermentable sugar in B might vary markedly from those of A/3.

The results are shown in Table V. The amount of fermentable sugar found in B is very much less than in either A or A/3. With reagent 1 the amount is less than one-half, in all but one sample. The use of reagent 2 shows 4 samples of B out of 6 to contain less fermentable sugar than A. The comparison of A and A/3 shows the latter in cc. 0.005 *N* thiosulphate to be almost exactly 1/3 the former.

Table V. *Showing the effect of the amount of dilution preliminary to the action of yeast and H_2SO_4 -Lloyd's reagent on fermentable sugar in normal fasting urine.*

Subject	Copper reagent 1			Copper reagent 2		
	A	A/3	B	A	A/3	B
R.W.I.U.	0.31	0.10	0.09	0.32	0.12	0.07
C.E.D.	0.28	0.10	0.04	0.30	0.10	0.09
C.E.D.	0.33	0.12	0.02	0.27	0.09	0.06
R.A.	0.40	0.13	0.06	0.41	0.12	0.08
D.L.S.	0.25	0.08	0.07	0.23	0.08	0.05
G.A.G.	0.42	0.12	0.04	0.27	0.08	0.10
Average	0.33	0.11	0.05	0.30	0.10	0.07
mg./2 hours as glucose	7.0	7.0	3.2	5.2	5.2	3.6

Results in cc. 0.005 *N* thiosulphate per 2 cc. urine-filtrate.

The error of simple dilution is 0.01 cc. Again the facts are inconsistent with the unity of the fermentable sugar fraction of normal fasting urine.

Why do the results with reagent 1 in the present work fail to duplicate our previous results, and why does reagent 1 give lower, similar and higher results with reagent 2 in Tables III, IV and V? Whilst we have not entirely excluded the possibility that small unconscious differences in detail in the use of the copper solutions or the preparation of the filtrates may be responsible for the discrepancies, it seems more likely that inequalities in the preparation of reagent 1 are the cause. The figures for fermentable sugar in fasting urine as obtained by reagent 2 are relatively stable from preparation to preparation, and each preparation can be easily checked by reference to the p_H or titration values of the B solution (Harding and Downs). Reagent 1 has also been standardised as far as possible, and each preparation is always checked in value against pure glucose and found to give the same standard figure. This, however, is known to be no guarantee that each preparation will possess the same value to all other reducing substances. Harding and Van Nostrand [1930] noted variations in oxidising power to non-sugar reducing substances of whole blood. Harding and Selby drew attention to the same fact, and this instability of the preparation was one of the reasons that caused us to seek a different copper oxidising reagent with good general properties. The instability is brought about in the preparation of the reagent by the necessity of neutralising Na_2CO_3 by free tartaric acid, with a resultant uncertainty of the carbonate-bicarbonate ratio, though this can be controlled with sufficient accuracy to obtain constant results with glucose (see criticisms by Somogyi [1926]). The results for fermentable sugar in normal fasting urine and after glucose, both morning and afternoon, as reported by Harding and Selby in their previous paper, were all obtained with one large preparation of the copper reagent. Two or three preparations have been used in the work of the present paper. If our explanation is correct, a somewhat accidental circumstance combined with our high preliminary dilution was responsible for our previous negative findings for fermentable sugar in normal fasting urine. On the other hand, all the evidence of the present paper makes uncertain the amount of glucose present in such urines and favours the minimum rather than the maximum figures.

Effect of oral administration of glucose on urine "fermentable" sugar.

We have re-examined this question, using conditions favourable to showing a high sugar removal by yeast.

50 g. glucose in 400 cc. water were taken orally at 9 a.m. under the same conditions as in our previous paper. Some days later 50 g. glucose were taken by the same subjects at 4 p.m. (under conditions of afternoon glycosuria), the noon meal being of general mixed character. Blood-sugars were estimated at 9 a.m., 2 p.m. and 4 p.m., and every 15 minutes after the glucose administration. We have reported only the zero, peak and 2 hour values. All blood-sugars were determined on cutaneous blood [Herbert and Bourne, 1930]. Urine was cleared by KH_2PO_4 - MgO followed by H_2SO_4 -Lloyd's reagent, and also by HgSO_4 - BaCO_3 . Yeast was used after clearing and the reducing power determined by reagent 2. The final dilution was approximately 1 : 10.

Table VI shows that there is a small rise in urine "fermentable" sugar after oral glucose administration, whether given fasting at 9 a.m. or at 4 p.m. even when the peak of the blood-sugar does not rise above the normally accepted renal threshold. When the peak rises above the usual threshold value, as in cases C.E.D. and A.R.A., the usual large amounts of fermentable sugar enter the urine. Unless this latter occurs, the increases, however, are so small that

Table VI. *Showing effect of oral glucose on urine "fermentable" sugar.*

Subject	Morning or afternoon	Blood-sugar, mg./100 cc.			Urine "fermentable" sugar mg./2 hours	
		0 hour	Peak	2 hours	Pre-glucose	Post-glucose
D.L.S.	M	77	124	97	5.0 3.5	6.4 6.8
	A	94	144	81	11.0 10.7	11.4 11.1
T.F.N.	M	87	130	85	3.8 —	4.5 —
	A	107	173	94	9.2 10.2	14.6 16.0
S.J.	M	80	132	66	2.7 3.5	7.0 6.1
	A	93	149	109	4.6 5.6	7.1 7.3
G.A.G.	M	90	134	82	5.8 5.5	7.0 6.7
	A	86	173	87	9.1 10.4	13.2 13.9
A.R.A.	M	107	150	60	4.7 5.0	6.1 6.1
	A	92	232	123	2.4 2.6	317 317
C.E.D.	M	97	154	91	4.1 4.4	5.2 5.0
	A	105	204	105	5.7 8.0	338 343

The first of each pair of urine "fermentable" sugar values is obtained after KH_2PO_4 -MgO followed by H_2SO_4 -Lloyd's reagent. The second of each pair is after HgSO_4 - BaCO_3 .

our previous conditions would have failed to detect them, unless we had adopted the extremely rigorous technique of Table I.

A correspondence between the blood-sugar level and the amount of urine "fermentable" sugar as determined after HgSO_4 - BaCO_3 or KH_2PO_4 -MgO and H_2SO_4 -Lloyd's reagent is also shown in Table VII. The morning blood-sugar

Table VII. *Showing relation of fasting blood-sugar to level of urine "fermentable" sugar.*

Subject	Blood-sugar 9 a.m. mg./100 cc.	Urine-sugar 7-9 a.m. mg./2 hr.	Blood-sugar 2-4 p.m. mg./100 cc.	Urine-sugar 2-4 p.m. mg./2 hr.
D.L.S.	77	5.0* 3.5†	115-94	11.0* 10.7†
S.J.	80	2.7* 3.5†	88-93	4.6* 5.6†
T.F.N.	87	3.8* —	122-107	9.2* 10.2†
G.A.G.	90	5.8* 5.5†	117-86	9.1* 10.4†
C.E.D.	97	4.1* 4.4†	124-105	5.7* 8.0†
A.R.A.	107	4.7* 5.0†	83-92	2.4* 2.6†

* Urine "cleared" by KH_2PO_4 -MgO followed by H_2SO_4 -Lloyd's reagent.

† Urine "cleared" by HgSO_4 - BaCO_3 .

at 9 a.m. is assumed as the level from 7-9 a.m. [Trimble and Maddock, 1929]. The blood-sugar level at 2 p.m. and 4 p.m. is usually higher than the fasting morning level. In all these cases the urine "fermentable" sugar is higher from 2-4 p.m. than from 7-9 a.m. The one exception where the 2-4 p.m. blood-sugar level is lower than in the morning shows a lower urine "fermentable" sugar.

The conclusion must be reached that fermentable sugar as obtained under such conditions is intimately connected with the level of the blood-sugar, or with the level of carbohydrate metabolism of which the fasting and post-prandial blood-sugar is also a reflex. In view of the facts set forth in the preceding sections showing the lack of unity of proof that glucose is the sole constituent of the fraction called urine "fermentable" sugar, we hesitate to conclude that the increases are glucose. The hesitation is strengthened if we tabulate the increases in urine "fermentable" sugar after glucose feeding in the order of the peaks of the blood-glucose. We should expect the two sets of figures to show some degree of correspondence if there is a simple escape of blood-glucose through the kidney. Table VIII shows such an arrangement. In making up the table

Table VIII. *Showing the peaks of blood-sugar and increase in urine "fermentable" sugar after glucose feeding.*

Blood-sugar peak mg./100 cc.	Urine "fermentable" sugar		
	Pre-glucose mg./2 hours	Post-glucose	Increase
124	4	6	2
130	4	4	0
132	3	6	3
134	6	7	1
144	11	11	0
149	5	7	2
150	5	6	1
154	4	5	1
173	9	13	4
173	9	15	6
204	7	340	333
232	2	317	315

we have taken the mean of the $\text{KH}_2\text{PO}_4\text{-MgO}$ and the $\text{HgSO}_4\text{-BaCO}_3$ values as representing the urine "fermentable" sugar to the nearest mg. It will be seen that no close correspondence between increases in blood-sugar and urine "fermentable" sugar exists. The increases after glucose feeding, though distinct, are small and remain so until, in this small series, the peak of the blood-sugar reaches 170 mg. The increase at this point is much more marked than after the lower blood-sugar peaks. At a peak of 204 mg. or 232 mg. the urine becomes flooded with fermentable sugar. This is the familiar description of the renal threshold for glucose, with a slight variation. Our figures supply no evidence for a gradual progressive increase in urine "fermentable" sugar with increasing peaks in blood-glucose.

West, Lange and Peterson evidently intend it to be understood that urine "fermentable" sugar as determined by their method is dependent on the level of the blood-sugar. They draw attention to a sharp increase in fermentable sugar in a dog's urine occurring after meat-feeding and remark, "it is probable also that the glucose of the blood reached a maximum about this time." Yet they ignore their findings that extremely irregular excretions of fermentable sugar apparently occur on two successive days of starvation in the same individual. Subject E.S.W. excretes 326 mg. fermentable sugar on normal diet, then 308 mg.

and 68 mg. on two starvation days. Subject H.L.W. puts out 38 mg. and 160 mg. fermentable sugar on two successive starvation days, the latter being slightly greater than after a day's normal diet. Such findings are inconsistent with a close correlation of blood-sugar and urine "fermentable" sugar. The blood-sugar during fasting exhibits no large fluctuations, such as are produced by the intake of food. Sugar remains at the normal fasting level with a slow fall as the fasting progresses from day to day.

SUMMARY.

1. Ammonium salts, in physiological amounts, lower the total "sugar" and non-fermentable "sugar" found in urine, when the determinations are made by some of the sensitive Shaffer-Hartmann reagents. The fermentable sugar value may be unaltered.

2. The use of KH_2PO_4 followed by MgO on normal urines constitutes a method of clearing urine. The filtrates are suitable for "sugar" determinations.

3. KH_2PO_4 - MgO can be used in conjunction with H_2SO_4 -Lloyd's reagent for clearing urines previous to "sugar" determinations.

4. The values for fermentable sugar in normal fasting urine can vary with the clearing agent and with the copper reagent.

5. All the variations of method permit of the recovery of added glucose to urine, in the form of extra fermentable sugar.

6. The amount of fermentable sugar found in normal fasting urine can be varied by the state of dilution previous to the application of H_2SO_4 -Lloyd's reagent.

7. Provided the method of clearing and the oxidising reagent correctly estimate small amounts of added glucose, the lower values for fermentable sugar should be accepted as more nearly correct.

8. Using the methods which give the higher results for fermentable sugar in urine, the oral ingestion of glucose results in slight but distinct increases in this fraction. These increases cannot be correlated with the increase in the blood-sugar. When the usual renal threshold for sugar is passed, large amounts of sugar appear in the urine.

9. We conclude that some substance removable by yeast other than glucose exists in normal human fasting urine.

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