LXXXV. THE CARBOHYDRATE OF NORMAL URINE.

By JOCELYN PATTERSON.

*From the Biochemical Department, Charing Cross Hospital Medical School,
London.*

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IN a recent paper Lund and Wolf [1925] show how the sugar (or sugars) of normal urine can be differentiated from glucose by the fact that, as a result of acting upon the urine in a Barcroft apparatus with a specially active yeast, carbon dioxide was evolved from specimens to which glucose had been added, but not from the urine itself. The first part of the present investigation shows that a very clear distinction may be made between the two by quite simple means.

If the urine is acted upon, in the presence of a small amount of toluene and a low concentration of phosphoric acid, by ordinary baker's yeast, the urine sugar is unaffected, whereas any small amount of added glucose is readily fermented with corresponding diminution in the true sugar content as estimated by Benedict and Osterberg's method [1918]. The investigation has been carried out, in order to simplify conditions, on the urines of persons on a moderate carbohydrate diet. The urinary sugar in cases where large quantities of carbohydrate are taken has not yet been fully examined. The loss of sugar previously ascribed to fermentation by Benedict and Osterberg [1918, 1921], who differentiated between fermentable and unfermentable sugars in normal urine, is evidently due to the action of organisms already present in the specimens or introduced along with the yeast. Indeed it is a well-known fact that the concentration of sugar in normal urine steadily diminishes on standing, without the addition of yeast.

To endeavour to show further differences between the urinary sugar and glucose, the attempt was made to isolate the former in the form of its osazone, and compare this with phenylglucosazone. This derivative was prepared by first treating the urine with Benedict's mercuric nitrate reagent to remove nitrogenous constituents, which seriously interfere with osazone formation, and glycuronic acid, and then applying the osazone-forming reagents in the usual way. The purification of the collected crystals was difficult, leading finally to a product which was evidently not quite chemically pure. It was without definite melting point, gradually decomposing between the temperatures 170–182°, passing into a viscous, tarry mass at 182–184°. Fig. 1 shows

the appearance of the urine sugar osazone when finally crystallised from boiling water, whilst Fig. 2 shows the appearance of phenylglucosazone obtained by an identical procedure. Owing to its marked insolubility in water, it is rather difficult to obtain glucosazone in good crystalline form from this medium, but it will be observed that under the conditions employed a characteristic shape is attained, although the individual crystals are considerably smaller than those usually obtained from dilute alcohol solution or from water containing an excess of phenylhydrazine.

The difference between the two substances is thus shown in most striking manner. Water is demonstrated to be much more suitable for the crystallisation of the urinary sugar osazone than for that of the glucosazone, owing to the greater solubility in hot water of the former. This fact has been used in devising fairly simple clinical laboratory tests, which it is hoped to publish shortly, to determine pathological sugar excretion in doubtful cases.

Fig. 1.
(Magnification 175)

Fig. 2.
(Magnification 175)

The elementary analysis of the urinary sugar osazone gave figures closely approximating to those calculated for a hexosazone. Unfortunately it was impossible to obtain a reading of the optical rotation. In attempting to apply the method of Nanji [1923] another difference between this osazone and most others of the sugar group was discovered. The *N* hydrochloric acid used in this method of determining nitrogen converts part of the material into a black friable humus-like mass, and the nitrogen value, obtained by subsequently

estimating the phenylhydrazine split off, was always considerably below that obtained by the combustion method.

In this investigation other forms of osazone have been met with in very small quantities, but in no case has it been possible to obtain any evidence of the presence of glucosazone. However, the compound described above is the derivative of what is undoubtedly the chief component of the sugars of normal urine.

The ease of decomposition of the osazone described above finds a parallel in the comparative instability of the sugar itself, as illustrated by the finding that, whilst in the majority of urines examined hydrolysis with hydrochloric acid (2 parts of concentrated acid to 100 parts of urine by volume) leads to an increase in the reducing sugar content through hydrolysis of dextrin-like carbohydrate, in not a few instances the reverse effect is obtained, indicating a destruction of the reducing sugar and the absence of hydrolysable dextrin to compensate. Where treatment with acid has led to an increase in free sugar, that portion liberated is for the most part fermentable under the same conditions as is glucose. In such cases the constant value attained after fermentation is at times below the initial before hydrolysis, again pointing to the partial destruction of the original reducing sugar.

EXPERIMENTAL.

All sugar estimations were made by Benedict and Osterberg's colorimetric method [1918].

Fermentation of normal urine.

To 100 cc. of urine were added 10 drops of toluene, 0.25 cc. of 85 % phosphoric acid and 20 drops of a thick suspension in saline of ordinary baker's yeast. Specimens were incubated at 37°.

Specimen	Before fermentation Sugar as glucose %	Time of fermentation hours	After fermentation Sugar as glucose %
1	0.090	24	0.087
2	0.032	24	0.033
3	0.040	17	0.035
4	0.100	17	0.102
5	0.090	17	0.084

Fermentation with added glucose.

0.1 g. of pure glucose was added to 100 cc. of urine which was then treated in the manner already described.

Specimen	Before fermentation Sugar as glucose %	Time of fermentation hours	After fermentation Sugar as glucose %
1	0.131	5	0.024
2	0.191	5	0.094
3	0.177	4	0.111

Isolation of the osazone.

Successive portions of 75 cc. of urine were treated exactly according to Benedict's technique for removing nitrogenous constituents and glycuronic acid, and in each case 100 cc. of clear colourless filtrate were collected after the separation of the excess zinc dust. This quantity was heated for $1\frac{1}{2}$ hours in a boiling water-bath with 2 g. phenylhydrazine hydrochloride, 6 g. sodium acetate, and 2 cc. glacial acetic acid. On allowing to stand overnight, crystals of osazone separated out and were collected from the several portions. In all 5 l. urine were treated in this manner and 1 g. crude osazone obtained. The crude product was crystallised twice from dilute aqueous alcohol, then twice from dilute pyridine and, finally, three times from hot water. For this purpose it was found most convenient to adopt a procedure somewhat similar to Soxhlet extraction, except that, instead of using the mid-piece of the Soxhlet apparatus, the thimble containing the substance was supported in the extraction flask above the level of the solvent in such a manner that the drops from the condenser fell upon the crystals at a temperature approaching 100° and filtration went on simultaneously with the hot extraction. Acicular crystals separated immediately on cooling.

The most highly purified material gave the following analytical figures:

	(1)	C	61.1 %	H	6.3 %	N	15.3 %
	(2)		60.9		6.3		15.5
A hexosazone requires			60.33		6.4		15.64

Fermentation of urine after hydrolysis.

Hydrolysis was performed with 2 parts concentrated hydrochloric acid per 100 parts of urine by volume for 15 mins. at 100° and fermentation carried out as described above.

(a) Hydrolysis increasing the reducing sugar content.

Specimen	Initial sugar (as glucose) %	Sugar (as glucose) after hydrolysis %	Sugar (as glucose) after hydrolysis and fermentation %
1	0.040	0.045	0.035
2	0.014	0.035	0.010
3	0.032	0.089	0.030
4	0.083	0.150	0.103
5	0.039	0.050	0.012

(b) Hydrolysis decreasing the reducing sugar content.

Specimen	Initial sugar (as glucose) %	Sugar (as glucose) after hydrolysis %	Sugar (as glucose) after hydrolysis and fermentation %
1	0.121	0.093	0.077
2	0.046	0.024	0.014

SUMMARY.

1. Under conditions in which glucose is readily fermentable the sugar of normal urine is completely unfermentable.

2. The osazone of the chief urinary sugar has been isolated, and marked differences between it and glucosazone noted with regard to crystalline form, melting point, solubility and stability towards acids.

3. The hydrolysis of the complex carbohydrate frequently found in normal urine liberates a reducing sugar which is fermentable.

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