ON THE
ABSENCE OF SUGAR FROM NORMAL
HUMAN URINE.

BY

GEORGE STILLINGFLEET JOHNSON, M.R.C.S., F.C.S.,
F.I.C.,

DEMONSTRATOR OF CHEMISTRY AT KING'S COLLEGE, LONDON.

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THE hypothesis that normal human urine contains sugar has on the whole been favoured more by chemists than by physiologists. The reason of this is that the chemical methods employed for detecting sugar depend for the most part upon the reducing action of that substance; and, until a comparatively recent date, no test for sugar was in common use which did not give more or less reaction with normal urine. Therefore, in the absence of a sufficient knowledge of other reducing agents in urine which react in the same way as sugar with the tests employed, it was concluded that sugar was invariably present in that secretion in small quantities.

I have convinced myself that sugar is absent from human urine by two methods :

1st. By studying the amount of reduction which normal urine produces ; by isolating the ingredients of normal urine which effect reduction in the same way as sugar, estimating their amount and their reduction equivalents ; and—

2nd. By applying to human urine a most sensitive test for sugar, with *which that secretion gives absolutely no reaction*. I will first give a brief sketch of the methods I have employed.

1st. Methods for isolating the reducing agents of normal urine, which resemble sugar in their action upon cupric oxide in boiling alkaline solutions.

Having observed the invariable presence of reducing agents in normal human urine, every specimen of which is found to exert more or less reduction of copper oxide in boiling alkaline solutions, I endeavoured to ascertain the nature of the substance or substances to which this reaction is due by the method of *precipitation*. The details of this investigation are published in the 'Proc. Roy. Soc.,' vol. xliii, pp. 493—534. Briefly the results obtained are as follows.

Precipitation with *mercuric chloride* removes from normal urine all substances which are capable of effecting reduction of copper oxide in the same way as glucose, but any glucose which may be present in diabetic urines is entirely unaffected by this treatment.¹ The proof that the reducing agents are removed by this method is that if the excess of mercury salt be subsequently precipitated by ammonia, the filtrate is destitute of reducing action. There are two substances in normal urine which resemble sugar in their reducing action upon cupric oxide : one a highly nitrogenous acid—*uric acid* ; the other a highly nitrogenous base— *kreatinin*.

¹ After adding a known quantity of glucose to a normal urine, then precipitating completely with mercuric chloride, I have found the glucose unaltered in the filtrate.

When mercuric chloride is added to urine the uric acid is precipitated immediately as mercury salt, and may be separated by immediate filtration; the kreatinin, however, is deposited very gradually from the filtrate in the form of a spherical mercury salt, forty-eight hours being required for complete precipitation.

About one-fourth of the reduction effected by normal urine is due to the uric acid which it contains, three-fourths to the kreatinin.

By weighing the mercury salt of kreatinin passed in twenty-four hours, analysing it, and finding that it contains about 20 per cent. of kreatinin, I have computed the daily output of this base by a healthy man at from 1·8 to 2·1 grammes in twenty-four hours.

Further, I have found that 12 grammes of this urinary kreatinin (a beautifully crystallised specimen of which I have brought for your inspection) are equivalent in reducing power to 10 grammes of glucose.

If we take 52·8 fluid ounces as the volume of urine passed in twenty-four hours, it follows that the reduction of copper oxide effected by the kreatinin of the urine is equivalent to that which would be produced by from 0·43 to 0·51 grain of glucose per 1 fluid ounce.

But the total reduction effected by normal human urine has been estimated as equivalent to 0·6 to 0·8 grain of glucose per fluid ounce, and one-fourth of this is due to uric acid; so that the entire reduction effected by the normal secretion is accounted for by these two substances.

My observation that one-fourth of the reduction effected by normal urine is due to uric acid confirms the work of Dr. F. W. Pavy, F.R.S. (vide 'Med.-Chir. Transactions,' vol. lxiii, p. 222). He found that precipitation of normal urine with lead acetate removed one-fourth of the reducing agents. I observed that the reduction of copper oxide by a normal urine always exceeds that effected by the same specimen upon picric acid (as applied by my father, Sir George Johnson), the ratio being as

4 : 3 ; and this discrepancy was traced to the fact that picric acid is not reduced by uric acid in boiling alkaline solutions, but it is reduced by the kreatinin.

As regards the reducing action of the urinary kreatinin, it was suggested by Dr. Oliver, in his 'Bedside Urine Testing,' that this base was responsible for much of the reduction effected by normal human urine upon cupric oxide.

Kühne also draws attention to the reduction of cupric oxide by kreatinin in boiling alkaline solutions in his 'Lehrbuch der physiologischen Chemie,' p. 505.

But the *amount* of reduction attributed to kreatinin by German workers is small ; thus E. Salkowski, in 'Centralblatt für die medicinischen Wissenschaften,' March, 1886, estimates the reduction due to uric acid and kreatinin taken together as varying from one-fifth to one-sixth of the total cupric oxide reduction effected by normal urine.

It remains, therefore, for me to explain the much greater reduction which I have observed in the kreatinin isolated by myself.

In its chemical relations kreatinin is closely allied to the vegetable alkaloids. The substance is, in fact, an animal alkaloid.

It is well known that substances of complex constitution are peculiarly liable to undergo isomeric changes under the influence of chemical agents, and especially when aided by heat ; so that after a lengthy and prolonged method of extraction has been resorted to, it does not by any means follow that the substance ultimately isolated is identical with that originally present.

I attribute the differences (which are many) between the kreatinin isolated by me from human urine, and the kreatinin prepared and isolated by other observers, to the fact that all through the series of processes which I have made use of, not only are violent chemical agents avoided as far as possible, but especially no heat is applied from first to last, so that I look upon my kreatinin as the actual base present in the urine—unchanged during extraction.

I think that this point will be made clear by a glance at the subjoined table from the 'Proc. Roy. Soc.,' vol. 1, p. 291.

Methods for Isolation of Kreatinin from Urine.

I. Heintz and Pettenkofer.	II. Liebig.	III. Maly.	IV. The Author.
Fresh urine neutralised with sodium carbonate, evaporated to a syrup. Syrup exhausted with alcohol, and alcoholic zinc chloride added.	Fresh urine neutralised with milk of lime. Calcium chloride added to complete precipitation. Filtrate evaporated till the salts crystallise out. Zinc chloride added to the liquor. Kreatinin zinc chloride dissolved in boiling water and treated with lead hydrate at the boiling temperature. Filtrate evaporated.	Urine evaporated to $\frac{1}{3}$ of its original bulk. Lead acetate added. Filtrate freed from lead by H_2S . Filtrate neutralised by sodic carbonate and precipitated by mercuric chloride. Precipitate suspended in water, and decomposed by H_2S . Filtrate evaporated. Residue recrystallised from alcohol.	Fresh urine + $\frac{1}{8}$ of its volume of cold saturated solution of sodic acetate, + $\frac{1}{2}$ of its volume of cold saturated solution of mercuric chloride. Filter immediately. Collect the precipitate which forms in the filtrate in 48 hours. Decompose the Hg salt by H_2S under water. Treat filtrate with $Pb(OH)_2$ at ordinary temperature. Evaporate filtrate <i>in vacuo</i> over H_2SO_4 .
	Product, kreatinin mixed with kreatine.	Product, kreatinin hydrochloride.	Product, efflorescent urinary kreatinin.

Differences between the kreatinin isolated by Author and the kreatinin described by Liebig :

	Kreatinin of urine.	Kreatinin (Liebig).
Solubility in water .	1 in 10·78 at 17° C.	... 1 in 11·5 at 16° C.
„ in alcohol	1 in 362 at 17° C.	... 1 in 102 at 16° C.
Platinum salt	$2(C_4H_7N_3O.HCl).PtCl_4.2H_2O$	... $2(C_4H_7N_3O.HCl).PtCl_4$.

The difference in reducing action is not more remarkable than some of the differences recorded in the above table.

So far I consider that the absence of sugar from normal human urine is proved, inasmuch as the entire reduction which the secretion can effect has been accounted for by two definite substances which have been

isolated from it, their amount therein ascertained, and their reducing power determined—viz. uric acid and kreatinin.

But an additional proof is afforded by the application of an extremely sensitive test for sugar, which gives no reaction whatever with the normal secretion of the human kidney; and this brings me to my second point.

2nd. The phenylhydrazine test for sugar.

C. Schwartz ('Pharm. Zeit.,' xxxiii, 465) describes the following test, which, though extremely sensitive in reaction with sugar, gives absolutely negative results with normal urine.

The urine is first precipitated completely with lead acetate and filtered, the filtrate is mixed with a solution of phenylhydrazine and excess of potassium hydroxide, and boiled for a minute. If sugar is present a yellow colour appears, followed by an orange precipitate on adding excess of acetic acid. The formation of this precipitate is more significant than the development of colour.¹

When this test is applied to normal urine no indication of sugar is obtained, and even the preliminary precipitation by lead acetate is unnecessary. For clinical purposes, however, it is better to precipitate first with lead acetate, since a urine loaded with urates may give a precipitate with acetic acid, which might be mistaken for the sugar precipitate. This is, I take it, the only reason for precipitating with lead acetate before applying the test, for uric acid gives no reaction with the phenylhydrazine.

It will be seen that we have now three tests for sugar in urine, which react as follows:

- (1) The cupric oxide test reacts with—
 - (a) Uric acid.
 - (b) Kreatinin.
 - (c) Glucose.

¹ The reason of this is that boiling with potassium hydroxide slightly deepens the colour of normal urine.

- (2) The picric acid test with—
 - (a) Kreatinin.
 - (b) Glucose.
- (3) The phenylhydrazine test responds with—
Glucose only ;

and the last gives absolutely no indication with normal urine, thus proving the absence of sugar from that secretion.

To ascertain whether anything present in normal urine interferes with the delicacy of the phenylhydrazine test, I have added known quantities of glucose to the secretion, and found that I could easily detect $\frac{1}{20}$ of a grain of glucose in one fluid ounce of urine.

In using the phenylhydrazine test for sugar, the crystalline phenylhydrazine hydrochloride should be employed in the proportion of four times the weight of the sugar present. In practice, as much as will lie on the end of a penknife is sufficient for one fluid drachm of urine. If sugar is present, even in so small a proportion as $\frac{1}{20}$ of a grain to the fluid ounce, the precipitate after acetic acid appears *within ten minutes*.

Finally I may refer to the clinical importance of the above facts. It is common, especially in the summer months, to meet with specimens of urine of high specific gravity (1030 and upwards), which give decided reduction of copper oxide, perhaps equivalent to from 1 to 1·5 grains of glucose per fluid ounce. Yet such urines are usually quite free from sugar. They are instances of so-called azoturia ; mixed with nitric acid and stirred, they generally deposit abundant crystals of urea nitrate ; and the other nitrogenous excreta, uric acid and kreatinin, being proportionately abundant, account for the increased reduction of cupric oxide. In such cases the phenylhydrazine test gives no indication of sugar, and at once proves the absence of that substance.

Appendix.

After an examination of the reaction of carbo-hydrates with benzoyl chloride, recommended to my notice by Dr. W. Hunter during the discussion on my paper, I see no reason to modify my statements as to the absence of glucose from normal human urine.

It is true that benzoyl chloride produces a precipitate when shaken with normal urine in presence of caustic soda, but as the test gives apparently identical results with glucose, starch, and many other non-saccharine carbo-hydrates, and as the phenylhydrazine test for sugar is so extremely sensitive, I consider that there is no reason to believe that normal human urine contains sugar.

Indeed, as a result of careful experiments conducted since my paper was read, I find that the phenylhydrazine test would detect sugar in the urine even if the quantity present did not exceed $\frac{1}{100}$ of a grain per fluid ounce.

Of course, for ordinary clinical testing, such delicacy of observation is unnecessary, the copper and picric acid tests being sufficiently sensitive for all cases.

(For report of the discussion on this paper, see 'Proceedings of the Royal Medical and Chirurgical Society,' Third Series, vol. v, p. 59.)