CCXIX. THE EFFECT OF HYDROLYSIS ON THE SUGARS OF NORMAL URINE.

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ACCORDING to various investigators, the reducing power of normal urine may, after acid hydrolysis, follow one of three courses. It may either diminish, increase or remain unaltered. If we accept the view that normal urines usually contain variable quantities of ill-defined polysaccharides, the augmentation of the reducing power after hydrolysis can be satisfactorily explained. The frequency and magnitude of the rise suggest that, under normal conditions, the excretion of such polysaccharides cannot be very large, although the polysaccharide content of the urine can be appreciably increased by the ingestion of certain carbohydrates such as dextrin. Folin and Berglund [1922] definitely demonstrated the presence of erythro-dextrin in the urine of a healthy individual after giving 200 g. of this material by mouth. In this particular case the increase in the reducing power after hydrolysis was very marked.

The explanation of the diminution of the reducing power after hydrolysis is, on the other hand, not so easy. Little is known about the nature of the non-carbohydrate reducing substances of urine apart from uric acid, urochrome and creatinine. There is reason to believe however that other reducing substances also occur. In the methods employed for the determination of the sugars of normal urine attempts are made, it is true, to remove these non-carbohydrate reducing substances but the inefficiency of these procedures is apparent when the methods are compared; no two methods agree when applied to one and the same urine. It follows that the diminution of the reducing power of normal urine, which sometimes occurs after hydrolysis, may be due to partial destruction of the sugars or of the non-carbohydrate reducing materials or of both. It is obvious that a diminution of the reducing power can be observed only in urines almost or completely free from polysaccharides. That such diminution is due to partial destruction of the sugars has often been suggested, but no direct experimental evidence, as far as we are aware, has been brought forward in support of this view.

Normal urine probably contains at least two sugars one of which is glucose whilst the other, which occurs in relatively greater proportion, has not yet been identified. This latter sugar will, for convenience, be called *isomaltose*, which name was given to it some time ago because of the occasional

similarity of its osazone to Fischer's *isomaltosazone*. Then, if we accept the view that the diminution of the reducing power is due to sugar destruction, it would be of interest to investigate the extent of the destruction as far as each sugar is concerned. This result should be more satisfactorily attained if some procedure were adopted by which the complete removal from the urine of the dextrin-like bodies and the other complex polysaccharides can be assured. The presence of these bodies must naturally disturb the result by masking the destructive action of the acid on the pre-existing sugars. Such mutually antagonistic effects must obtain in urines in which the total reduction is not altered by hydrolysis.

While it is possible to devise a means for the complete removal of the complex carbohydrates, the removal of sucrose is not so easy. Fortunately, however, sucrose is not frequently met with in the urine, besides which it is possible to limit the examination to urines of subjects known not to have partaken of foodstuffs containing this disaccharide.

Working on these lines and with the help of the osazone reaction it has been possible to demonstrate that hydrolysis by acid destroys a large part of the sugar called *isomaltose* while glucose seems to be unaffected.

EXPERIMENTAL.

All the urines investigated were collected from young healthy individuals and in every case the food taken by the subject was noted. About 150 samples were examined and these represented odd specimens as well as 24 hour collections.

Every sample was first treated with charcoal, this treatment being an essential step in the preparation of the osazones of the urine-sugars. However, it must be remembered that charcoal not only removes the substances which normally interfere with the proper crystallisation of the osazones, but unfortunately also removes a variable quantity of the urine-sugars. After having compared the action of five specimens of charcoal, it was found that the B.D.H. decolorising charcoal was most suitable, since it interfered least with the urine-sugars and gave a clear colourless filtrate.

The charcoal treatment consisted in adding 8 g. charcoal to 100 cc. urine and filtering after allowing the mixture to stand for 5 minutes. The clear colourless filtrate was employed for the determination of the reducing power and the preparation of the osazone both before and after hydrolysis.

The hydrolysis of the charcoal filtrate was done with the help of HCl in a manner similar to that recommended by Folin and Berglund [1922], 20 cc. were measured in a tube graduated for the purpose, 2 cc. 10 % HCl were then added and the contents heated in a boiling water-bath for 75 minutes. After thorough cooling, the hydrolysed filtrate was neutralised with a few drops of a strong solution of NaOH. At first phenolphthalein was used as an indicator but later its use was discontinued and the alkali was very carefully added till

(a)

(b)

Osazones obtained from the same urine filtrate.
(a) Before, (b) after hydrolysis.

the phosphate precipitate just began to form. Then water was added to bring the volume back to the 20 cc. mark.

The reducing power of the filtrate was determined by Shaffer and Hartman's method [1920] and in the preparation of the osazone the method described by Hassan [1928] was followed.

Changes in the reducing power.

As has been stated previously about 150 urines were examined and with the exception of three, all showed a marked diminution in the reducing power after hydrolysis. Table I gives the figures obtained in a few cases.

Table I. *Effect of hydrolysis on reducing power.*

Total reduction in mg. glucose per litre	
Before hydrolysis	After hydrolysis
412	350
322	150
266	124
120	34
370	350
158	132

The samples which showed an increase after the acid treatment constituted 2 % of the total and in all sucrose was proved to be present. As a matter of fact the subjects from whom these three specimens were obtained admitted partaking of meals containing pastries sweetened with cane sugar syrup.

The causes of this diminution may be numerous, but the present work has been limited to investigating the influence of the acid on the urine-sugars. It was thought that the osazone reaction would be helpful in this respect. This reaction is not only capable of demonstrating changes in the quantity of the sugars but it can also show, within reasonable limits, variations in their proportions. The osazones of the sugars of the charcoal-treated filtrate have therefore been prepared before and after hydrolysis and the products compared.

In order to have a clear idea of the following comparison, the reader is referred to the paper by Hassan [1928] in which a description of the osazones of the sugars of normal urine is given. There it is shown that these compounds are crystal mixtures formed of at least two components, and that, under suitable conditions, these crystal mixtures can be split into two osazones having distinct characters. Osazone I crystallises out in rosettes formed of broad flat needles, is soluble in hot water and melts at about 152°, and osazone II is identical with glucosazone.

On comparing the microscopic appearance of the two products, a striking difference was immediately noticed. The osazones before hydrolysis presented the usual crystal mixtures in which the proportions of the two osazones varied greatly. Those obtained after hydrolysis showed, on the other hand, a marked diminution in the broad needles and a tendency to aggregate in sheaf formations instead of the usual rosettes and in a large number of the samples the crystal mixtures were replaced by glucosazone sheaves (see Plate VIII).

This difference can only be due to one of two factors. Either hydrolysis increases the glucose content of the filtrate or destroys the urine-sugar which gives rise to osazone I. In the former case the glucosazone character will predominate by virtue of the increased concentration of glucose, while in the latter the destruction of the isomaltose will leave the field free to the pre-existing glucose. It can, however, be argued that the change in the crystal shapes might simply be due to changed conditions of crystallisation and not to an alteration in the proportions of the two sugars. This objection is quite reasonable because it is very unusual, when the osazones of the sugars of any one urine are recrystallised, to get the same crystal shapes. The crystal mixtures obtained on recrystallisation can assume a large variety of appearances depending on the rate of crystallisation and so forth, but it is always possible to see that in the new crystal mixtures the proportions of the two components remain practically the same. The difference cannot be due to the formation of new sugar because the charcoal employed adsorbs the dextrin-like bodies, at least in the concentration in which they occur normally. Moreover the result reported above, concerning the uniform diminution of the reducing power after hydrolysis, is in itself a proof that the charcoal-treated filtrates are free from compounds capable of giving rise to fresh reducing substances on hydrolysis; sucrose is, of course excluded.

The experiment described above shows that the diminution in the reducing power and incidentally the change in the appearance of the crystal mixtures are probably due to destruction of isomaltose, *i.e.* the sugar which gives rise to osazone I. If this conclusion is correct then we should observe the following changes in the osazones of the hydrolysed filtrates.

- (a) The osazone yield should be less.
- (b) There should be a rise in the M.P. of the crystal mixtures.
- (c) The solubility of the crystal mixture should be less.

If destruction of the sugars is taking place during hydrolysis, one naturally expects to get less osazone formed after the acid treatment. To show that this is actually the case is by no means easy because of the difficulty of purifying the small yield without incurring great loss. After many trials we adopted the following procedure which, though it cannot give a pure product, is quite adequate for comparative purposes. The osazones were prepared under exactly similar conditions from the filtrates before and after hydrolysis. The precipitate was then filtered in a weighed Gooch crucible, washed with cold water and treated with as little chloroform as was necessary to wash away the oxidation products of the phenylhydrazine. The same volume of chloroform was used in both cases and the washing was stopped when the chloroform was free from colour. The crucible and contents were then dried to a constant weight. In all cases the osazone yield weighed less after hydrolysis (see Table II). The difference, however, did not always run parallel with that found for the reducing power. A possible explanation of this divergence will be discussed later on.

Table II. *Osazone yield.*

Weight of osazone in mg.	
Before hydrolysis	After hydrolysis
2.4	2.0
3.8	1.0
4.8	2.0
5.0	2.6
4.6	3.0

The melting-point and solubility.

Crystal mixtures vary in their m.p. and the variation depends on the proportion of each sugar present. Those containing more glucosazone would be expected to have a higher m.p. than those with relatively more of osazone I. If hydrolysis destroys the sugar giving rise to osazone I, that is, the osazone with the lower melting-point, a rise in the m.p. of the resulting osazone should follow. To prove this, an adequate quantity of the charcoal filtrates (50 to 100 cc.) was used for the preparation of the osazones and the melting-point of the crystals, purified by recrystallisation was determined. The following figures represent some of the results obtained.

Melting-point determinations	
Before hydrolysis	After hydrolysis
162°	181°
163°	186°
161°	180°

As would be expected the solubilities of the products exhibited unmistakable differences. In any one urine, the osazones obtained before hydrolysis were more soluble in hot water and in cold alcohol than those obtained after hydrolysis.

DISCUSSION AND CONCLUSIONS.

Whether *isomaltose* or some other compound gives rise to osazone I, the parent substance seems to be slowly destroyed by acid hydrolysis, whilst glucose is unaffected under the same conditions. This assumption is supported by the microscopic appearance of the crystal mixtures in which an increase rather than a decrease of the glucosazone element is noted after hydrolysis. This increase is, however, only apparent, for the diminution in osazone I will not only give the glucosazone a chance to impress more of its own characters on the crystal mixtures but may, if the diminution is great, even allow it to form typical glucosazone. Moreover, subjecting an aqueous solution of glucose containing 40 mg. per 100 cc. to acid hydrolysis did not affect its reducing power. The destruction of *isomaltose* does not account, in many cases, for the total diminution in the reducing power and, as a matter of fact, there is no reason to believe that it can. Even in the charcoal-treated filtrate, the sugars are not the only reducing substances present. The lack of parallelism, referred to above, between the diminution in the reducing power and the

weight of the osazone found after hydrolysis points to the possible existence in the filtrate of non-carbohydrate reducing substances, and it is highly probable that some of these substances undergo destruction by acid as well.

SUMMARY.

1. The reducing powers of the charcoal-treated urines of about 150 healthy subjects were determined before and after hydrolysis with 10 % hydrochloric acid.

2. With the exception of three cases, in which sucrose occurred, a diminution in the reducing power was observed.

3. Evidence is given to show that this diminution is due, at least in part, to destruction of the so-called *isomaltose* occurring normally in urine.

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