ON THE INFLUENCE OF KREATININ IN MODIFYING CERTAIN REACTIONS OF SUGAR IN URINE

By HUGH MACLEAN, M.D., *Lecturer on Chemical Physiology, University of Aberdeen.*

From the Physiological Laboratory, Aberdeen University

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In a former article¹ it was pointed out that the principal normal constituent of urine which markedly interferes with the results obtained on applying Fehling's test for the detection of small amounts of sugar is kreatinin. The constant presence of kreatinin in urine completely invalidates the test when used in the ordinary way for the detection of minute amounts of sugar, and it is probably for this reason that the constant physiological occurrence of sugar in urine has for so long been doubted or denied. Kreatin acts in the same way only it is much less powerful. Since, according to Folin,² kreatin may appear in the urine in large amounts, especially after a meat diet, it is likely that in certain urines kreatin may largely enhance the effects of kreatinin; in the majority of urines, however, the amount of kreatin appears to be small and its direct effect of minor importance.

Kreatinin is capable of acting in different ways when heated with Fehling's solution to which has been added small quantities of some reducing agent such as sugar. It is itself capable of slowly reducing Fehling's solution and at the same time possesses the property of holding the reduced suboxide in solution, thus apparently inhibiting its own reducing action as well as that of any other reducing constituent that may be present.

When, as often happens in urine, the amount of kreatinin present is barely sufficient to hold in solution the reduced suboxide obtained by the action of small amounts of sugar as well as by that of the kreatinin itself, the result is so modified as to render its interpretation doubtful. Those ambiguous reactions in which a more or less opalescent solution tending immediately or after cooling to show an indistinct greenish-

1. *Bio-Chemical Journal*, Vol. I, p. 111.

2. *Zeit. f. Physiol. Chem.*, Bd. XLI., S. 223 (1904).

yellow precipitate suspended in the liquid, have hitherto constituted one of the chief objections to this otherwise excellent test ; if, however, the above explanation of this phenomenon be kept in view it may help in deciding what value is to be attached to this characteristic reaction in any individual case. In the absence of drug treatment these opalescent solutions generally indicate the presence of sugar in amount somewhat beyond the normal percentage ; it is obvious that the amount of urine actually passed per diem must be taken into account in estimating the significance of such reaction. On the other hand, the absence of kreatinin or some retarding factor in urine would also interfere with Fehling's test ; for the normal amount of uric acid alone, exclusive of other reducing agents present, gives a fairly marked Fehling's reaction.

ON THE MANNER IN WHICH KREATININ INHIBITS THE USUAL EFFECT OF SMALL QUANTITIES OF SUGAR ON FEHLING'S SOLUTION

While it seems certain that the only important nitrogenous constituent of normal urine that exerts a marked influence in retarding the effects of Fehling's solution is kreatinin, there are obviously two ways in which this might be accomplished : (1) the alkaline part of Fehling's solution might act on the kreatinin and generate ammonia, which, if evolved in sufficient quantity, would hold the reduced suboxide in solution ; or (2) the kreatinin, as such, possesses the property of holding the suboxide in solution.

That the first hypothesis is incorrect is evident from the following considerations and experiments.

(a) About 2.5 mgr. kreatinin is required to prevent precipitation of 1 c.c. of .1 per cent. aqueous dextrose solution when mixed with 1 c.c. Fehling's solution. If we assume for purposes of experiment that all the nitrogen of the kreatinin goes to form ammonia, the total amount of NH_3 resulting from the decomposition of this amount of kreatinin is only about 1.12 mgr.

$$\text{C}_4\text{H}_7\text{N}_3\text{O} = 3(\text{NH}_3) = \frac{51}{113} \times \frac{5}{2} = 1.12 \text{ mgr.}$$

(M.W. 113) (M.W. 51)

A reference to the following tables shows that a much greater amount of ammonia than 1.12 mgr. is necessary to prevent the appearance of a precipitate with a solution of .1 per cent. dextrose.

TABLE I (AMMONIA)

	Ammonium hydrate pur. ('880) (1 in 10)	Ammonia calculated as nitrogen in mgrs.	Results
1 c.c. .1 % dextrose solution + 1 c.c. Fehling's solution boiled with	1 drop	1.5 mgr.	Immediate precipitate
	2 drops	3.0 "	" "
	4 "	6.0 "	" "
	6 "	9.0 "	pp. after few seconds boiling
	8 "	12.0 "	" " "
	10 "	15.0 "	pp. after 6-10 seconds boiling
	12 "	18.0 "	pp. after 8-12 " "
	16 "	24.0 "	pp. after little time

In these cases the cause of the precipitate being produced after boiling for a few seconds was not due to the fact of the ammonia being driven from the hot liquid, for ammonia still continued to come off in great abundance long after a distinct precipitate had formed. It will be noticed that such a large amount as 24 mgrs. of ammonia is insufficient to prevent a reaction for more than a few seconds with .1 per cent. dextrose solution, whereas 2.5 mgrs. kreatinin, with a total possible evolution corresponding to 1.12 mgr. NH_3 , entirely prevents precipitation even after fairly prolonged boiling.

TABLE II (KREATININ)

	Kreatinin	Total N calculated as ammonia	Results
1 c.c. .1 % dextrose solution + 1 c.c. Fehling's solution boiled with	1 mgr.	0.45 mgr.	Opalescent solution
	2 mgrs.	0.90 "	Slight reaction after half minute
	2½ "	1.12 mgrs.	No pp. after long boiling (one minute)
	3 "	1.35 "	" " "
	4 "	1.80 "	" " "
			sol. slightly greenish-blue

(b) Several mixtures of kreatinin and alkaline part of Fehling's solution were distilled for periods varying from three to eight minutes and the ammonia calculated in the ordinary way by $\text{N}/_{10}\text{NaOH}$;

after several minutes boiling the amount of ammonia did not nearly correspond to the total amount of nitrogen present in the kreatinin, the quantity evolved being absolutely insufficient to exert any inhibiting influence on even a minute quantity of sugar; a certain amount of ammonia was always obtained, but it was evolved very slowly and in small quantity.

(c) An examination of the liquid used in the above experiments showed that it still possessed a fairly marked inhibitory effect after five minutes boiling; this proved that the kreatinin had not been all destroyed in this time.

(d) Distillation for several minutes of small quantities of normal urine mixed with alkali, and giving a strong kreatinin reaction, resulted in the production of ammonia in quantity insufficient to exert any marked retarding effect.

From these observations it seems obvious that the influence of the alkali of Fehling's solution on the nitrogenous constituents of the urine generating free ammonia is quite insufficient to explain the retarding effects described above; any ammonia generated undoubtedly helps, but only to a limited extent, for from its slow evolution there cannot be more than a comparatively small quantity present in the boiling liquid at any particular moment. Urea in the percentage in which it occurs in urine yields much more ammonia than the kreatinin of an equal amount of urine, and yet it possesses no apparent retarding effects; for a very dilute solution of dextrose (less than 0.1 per cent.) introduced into a 2.3 per cent. urea solution and mixed with equal parts of Fehling's solution gives quite a distinct reaction. Kreatinin therefore acts by holding the reduced suboxide in solution.

ON THE MODIFICATION OF COLOUR OBTAINED BY FEHLING'S TEST IN URINE CONTAINING SMALL AMOUNTS OF DEXTROSE

If a weak solution of dextrose (say 0.4 per cent.) in distilled water is boiled with an equal amount of Fehling's solution a *red* precipitate of cuprous oxide is quickly deposited; if the same amount of sugar be added to normal urine and Fehling's solution used as before the resulting precipitate is distinctly *yellow*, and differs markedly in appearance

from that obtained from dextrose in distilled water; if a little kreatinin be added to watery sugar solution a yellow precipitate is obtained, exactly like that obtained from the urinary solution. This modification in colour is generally admitted to be caused by kreatinin, and is generally explained in the text books as being due to a deposition of cuprous hydrate ($\text{Cu}_2\text{OH}_2\text{O}$). A quotation taken at random from a popular clinical text book is as follows: 'On boiling, the blue cupric hydrate is reduced to cuprous hydrate, which is not capable of being held in solution and accordingly appears as a yellow precipitate, which may be further dehydrated to cuprous oxide (Cu_2O) which is red.' This explanation seems to be generally accepted, it being assumed that a yellow precipitate indicates the lower *hydrate*, whereas a distinctly red one indicates the lower *oxide*. Thus, almost every urine containing enough sugar to give a precipitate with Fehling's test, apparently gives the yellow hydrate. The lower hydrate of copper is, however, one of the most unstable of chemical compounds, and it appears strange that it should appear so frequently in urine testing with Fehling's solution. This yellow substance is by no means unstable, for it often remains yellow for hours or even days, as can be easily observed if some of it be poured into a quantity of distilled water and put aside; the ordinary cuprous hydrate under similar conditions very soon changes, and this at once suggests a doubt in regard to the constitution of the yellow precipitate obtained from urine.

It would appear that both the red and yellow precipitate are really the same chemical substance—the lower oxide of copper (Cu_2O).¹ The difference in colour does not necessarily indicate a change in the chemical composition, but seems to be dependent on the state of subdivision of the precipitate; if the precipitate is finely subdivided it has a yellow colour; if comparatively coarse it is red. This can be demonstrated by examining different precipitates under the microscope. The red precipitate is seen to be much more coarsely granular,

1. As far as I am aware the first to suggest that the yellow precipitate obtained on heating certain saccharine urines with Fehling's solution is probably not the cuprous hydrate, but really the modified cuprous oxide, was Dr. Fraser of the Aberdeen Royal Infirmary, who informs me that he has taught this for many years.

being quite different in this respect from the yellow. If the red oxide be taken and thoroughly dried and then well ground in a mortar, it gradually assumes a yellow appearance; on subsequent mixture with water a yellowish fluid is obtained. The red precipitate can be comparatively easily separated from the urine by filtration, but often it is very difficult to separate a markedly yellow one owing to the fineness of the particles. If the precipitate is very finely divided it appears as a greenish opalescence which may take many hours to fall down, and even by centrifugalising it is difficult to separate it from the liquid in which it is suspended; a distinctly yellow precipitate, on the other hand, can be fairly easily thrown down by the centrifuge, and the red one separates out very readily indeed. If a few drops of urine giving, when tested in the ordinary way, a yellow precipitate, be dropped into a fairly large quantity of boiling Fehling's solution, a red precipitate separates out—in other words, a urine giving a yellow precipitate when heated with equal parts of Fehling's solution gives a red precipitate when heated with excess.

All the above modifications are explained by the fact that the state of subdivision of the precipitate of cuprous oxide obtained when a weak solution of dextrose, or urine containing a small percentage of sugar, is heated with Fehling's solution depends on the relative amount of kreatinin present. When the amount of the latter is just insufficient to inhibit the influence of the sugar present, a very finely divided precipitate of cuprous oxide separates out, which may show merely as an opalescent milky solution; the addition of just a trace of sugar to the solution makes it still more difficult for the kreatinin to hold the suboxide in solution, and it is deposited in a slightly coarser form than before, as indicated by an opalescent solution in which the eye can detect a faint precipitate on careful observation; at this stage the general colour of the solution is a dirty greenish yellow; the addition of extra traces of sugar makes the precipitate coarser and coarser, and soon a markedly yellow liquid showing a distinct precipitate is obtained. If still more sugar be added a red precipitate appears, which soon settles to the bottom of the tube, and is seen by microscopic examination to be fairly granular.

The following table indicates the changes observed in the precipitate obtained from a urine, to which different strengths of sugar has been added.

Amount of sugar added per cent.	Time required for boiling	Appearance	Time required to separate precipitate by centrifuge ¹	Microscopical appearance	Filtration
Trace	2½ minutes	Greenish opalescence	2 hours	Very many minute particles with few large ones	Impossible
·015 %	1¼ „	Greenish pp	1 „	„	Extremely difficult
·04 %	1¼ „	Greenish-yellow pp.	½ hour	Many fine : fair number coarse	Very difficult
·1 %	30 seconds	„	10 minutes	„	Fairly difficult
·4 %	10 „	Yellow pp.	10 „	Mixture of very fine and fairly coarse	Rather difficult to get quite clear solution
3·0 %	Immediately	Red pp.	2½ „	Large, coarse, with comparatively few fine granules	Easy to get clear filtrate

By carefully adjusting the relative amounts of kreatinin and sugar present in a liquid all shades of colour from greenish-yellow to bright red can be obtained after boiling with Fehling's solution. A small quantity of sugar (say ·15 per cent.) added to distilled water gives a more or less dirty yellow appearance, but this is caused by the red particles floating in the blue liquid, and here there is really no fine subdivision, for on centrifugalising the liquid a bright red precipitate is obtained, and the blue liquid appears quite free from suspended matter; the centrifuge completely separates the precipitate in a very short time, and in fact, if left to stand, it generally falls in from five to ten minutes. A trace of kreatinin added previous to boiling results in the precipitate being quite yellow as described above.

Thus it would seem that here we have an explanation of all the

1. The centrifuge used was purposely not a rapid one.

different coloured precipitates obtained on boiling urines containing small amounts of sugar with Fehling's solution. In urine the first part of the suboxide that separates out is exceedingly fine; as the kreatinin loses its effect the precipitate becomes more coarsely granular, till ultimately the ordinary red is obtained. In urine we have the results of all these stages depending on the relative amounts of sugar and kreatinin present, and thus the colour shows notable differences.

In order to demonstrate the different stages it is only necessary to add a trace of sugar to urine and boil till the first sign of opalescence is detected. Then centrifugalise for some hours and add a second trace of sugar to the blue liquid obtained; heat, and again centrifugalise. This time the precipitate obtained is slightly more yellow than at first, and if this be repeated for a few times the precipitate will ultimately appear quite red. Exactly the same phenomena can be seen with kreatinin itself. Kreatinin tends slowly to reduce Fehling's solution and after some time an opalescent, slightly greenish, milky liquid is obtained; filtration results in the filter paper being stained green, the filtrate, however, being still opalescent. If boiling is continued for some time longer and the liquid again filtered, the stain is now seen to be distinctly yellow; and a third filtration, after somewhat prolonged boiling, results in the filter paper being distinctly red. The following are notes of an experiment with kreatinin.

TABLE IV

Weak solution of kreatinin with equal parts of Fehling's solution	{	Boiled for 5 minutes = greenish opalescent fluid; after filtering still opalescent, but filter paper stained greenish.
		„ 6 minutes = Filter paper stained greenish- yellow; liquid still opalescent.
		„ 10 minutes = Filter paper stained distinctly red, showing granular pp. of Cu_2O . Filtrate still opalescent.

THE SODIUM-NITRO-PRUSSIDE TEST AND THE PICRIC ACID
TEST FOR KREATININ

In performing experiments to determine the reduction of kreatinin after boiling in alkaline solution, it was necessary to make use of some fairly delicate indicator. Picric acid in alkaline solution when used in the cold, while very definite in regard to strong kreatinin solutions, was found to be worthless for small amounts as no reaction was obtained ; on the other hand, it was equally unsatisfactory after heating, for a mixture of picric acid and alkali, in the absence of all other substances, gives on heating a more or less distinct red colour. Nitro-prusside of sodium in alkaline solution was found, however, to be a very delicate test, and with care such a small quantity as $\cdot 012$ per cent. kreatinin in solution could be detected with certainty. It is generally stated that the test is not applicable to a solution containing less than $\cdot 03$ per cent. kreatinin, but by the use of strong solution of sodium-nitro-prusside there was no difficulty in detecting even less than $\cdot 012$ per cent., especially when a control test with a solution of alkali and nitro-prusside without kreatinin was made use of.

This reaction of nitro-prusside of sodium with kreatinin in alkaline solution was pointed out by Weyl¹ many years ago. Salkowski² showed later on that the addition of acetic acid to the red coloured liquid obtained by Weyl's reaction, and boiling, it turns greenish or greenish-blue, and on standing, Prussian blue separates. The application of this reaction as a test for acetone has been utilised by Legal³ and Le Nobel,⁴ and a considerable amount of confusion seems to prevail in regard to the exact reactions given by kreatinin and acetone respectively. That the addition of a few drops of sodium nitro-prusside and sodium hydrate to a solution containing either acetone or kreatinin will in each case produce a ruby-red colour, which changes to yellow on standing or heating, is generally acknowledged, but the

1. *Berichte, der Deutsch. Chem. Gesellsch.*, 1878, p. 228.

2. Salkowski, *Zeitschr. f. Physiol. Chem.*, 1880.

3. *Pharm. Journal* [5], XVIII, 206.

4. *Chem. Centralb.*, 1884, p. 626.

subsequent action of acidulation and heat has given rise to many apparently contradictory statements.

In regard to acetone, Legal¹ states that a little acetic acid added to the yellow liquid produces a deep violet colouration.

According to Le Nobel,² a greenish-blue or violet colour is developed on boiling after the addition of an acid.

Allen³ states that 'acetone gives a ruby-red colour with Weyl's test, though no blue colour can be obtained on acidulation, acetic acid merely restoring the yellow to red.'

Milroy⁴ states that acetone and kreatinin give a somewhat similar reaction with Weyl's test.

Dixon Mann⁵ says 'acetone gives a red colour with Weyl's test but not the subsequent blue.'

In another text book⁶ the following statement is made, 'acetone gives a red colour with the nitro-prusside and alkali which, however, does not become yellow on standing or even after adding acetic acid.'

In performing Weyl's test for kreatinin it is well known that the red colour obtained changes very quickly to yellow on the addition of heat: in applying the test it is very important that the liquid be not subjected to heat *before* adding acetic acid as is sometimes done, for the nitro-prusside of sodium itself, when heated in the presence of an alkali, undergoes a change and yields a very marked greenish or greenish-blue solution on the addition of a small quantity of acetic or other acid. The marked greenish-blue colour so easily obtained on the addition of acetic acid to urine which has been heated with sodium nitro-prusside and an alkali, depends largely on the interaction between these reagents, and is not simply due to the influence of kreatinin. This action of sodium nitro-prusside and alkali seems to afford an explanation of the contradictory nature of many statements made in regard to the acidulation and heat part of this test.

1. Loc. cit.

2. Loc. cit.

3. 'Chemistry of Urine,' 1895, p. 158.

4. 'Practical Physiological Chemistry,' 1904, p. 106.

5. 'Physiology and Pathology of the Urine,' 1904, p. 47.

6. 'Practical Physiology' (Beddard, Edkins, Hill, Macleod, Pembrey) 1902, p. 261.

That heat is sometimes applied before the addition of the acid is amply proved by the statements of some investigators.

Tests performed with a chemically pure sample of acetone showed that acetone and kreatinin gave practically the same result so far as the nitro-prusside and alkali reaction is concerned—a ruby-red colour gradually changing to yellow. With a comparatively dilute solution of acetone the addition of a few drops of strong acetic acid gives, on agitation of the liquid, a distinct purplish colour; this colour is destroyed by heat, and a clear or slightly greenish-blue liquid is left, which gradually becomes of a deeper tint. The addition of excess of acid tends to destroy this purple or violet colour; subsequent heating, however, still gives a greenish colouration.

With a stronger solution of acetone (say 5 per cent.) the addition of a comparatively large amount of acetic acid does not destroy the violet colour produced; on heating, however, it is very quickly destroyed, and a very marked greenish-blue colour takes its place. Thus it is obvious that both kreatinin and acetone give practically the same result in regard to the formation of a greenish-blue or blue liquid.

The only difference in the test is that a fair amount of acetic acid added to the yellow liquid obtained in the second stage of the nitro-prusside reaction, gives, in the case of kreatinin, a *clear amber colour* (provided care is taken that the mixture be not previously heated), whereas with acetone a *purple* or *violet* colour is produced which, in weak solutions, tends to be destroyed by excess of the acid.

Weyl's reaction is, of course, positive in every urine on account of the kreatinin present, but a little care in the application of the test renders the detection of acetone comparatively easy. The red coloured liquid obtained after alkaline nitro-prusside should be slightly acidified with acetic acid and shaken, and if a clear light-amber coloured liquid is now obtained the red colour is due to kreatinin; if the acidified liquid is slightly red or purple acetone is present as well. Heating in each case gives the same result—a green or greenish-blue liquid.

THE ACTION OF THE ALKALINE PART OF FEHLING'S SOLUTION
ON KREATININ

To determine the changes which kreatinin undergoes when boiled with Fehling's solution, a definite amount of kreatinin was boiled for varying periods in a 4 per cent. sodium hydrate (watery) solution. Samples of the boiled liquid were obtained, and of these 1 c.c. was taken and four drops of a 15 per cent. solution of nitro-prusside of sodium added, and the mixture shaken. Numerous tests were made in order to ascertain how many drops of the original kreatinin solution (made up to 1 c.c. with 4 per cent. sodium hydrate solution) gave the same colour, and faded at the same time as the boiled solution.

As an example, it was found that two drops of a .1 per cent. solution of kreatinin added to fourteen drops of 4 per cent. sodium hydrate solution (sixteen drops from the pipettes used exactly corresponded to 1 c.c.) gave with nitro-prusside of sodium a red colour which faded in about twelve seconds. This .1 per cent. solution was boiled for about eighteen minutes, and it was then found that 1 c.c. of it gave a red colour similar to the original solution, this red colour fading in about twelve to fifteen seconds. From this it was obvious that 1 c.c. of the boiled kreatinin solution contained the same amount of kreatinin as two drops of the original solution. Two drops of the original solution contained .125 mgr. kreatinin; therefore, by boiling for eighteen minutes the kreatinin solution was reduced from a strength of 1 mgr. per c.c.—a reduction of about 85 per cent. The following notes of one of the experiments show the general change.

TABLE V

Kreatinin sol. 50 mgr. in 50 c.c. water + 2 grms. sodium hydrate

Time boiled		Amount (in drops) of original solution made up to 1 c.c. with 40 per cent. sodium hydrate giving reaction similar to 1 c.c. boiled kreatinin solution		No. of mgrs. present in 1 c.c. of kreatinin solution after boiling (original solution = 1 mgr. per c.c.)
3 minutes	...	9-10 drops	...	0.60 mgr. per c.c.
6 "	...	6-7 "	...	0.40 " "
10 "	...	3-4 "	...	0.23 " "
12 "	...	3 "	...	0.20 " "
17 "	..	2-3 "	...	0.16 " "
25 "	...	1-2 "	...	0.1 " "
30 "	...	Trace	...	—

Similar experiments bear out the above results, viz.:—that about 50 per cent. of a weak kreatinin solution is changed after boiling from four to seven minutes in the presence of caustic alkali in half the strength present in Fehling's solution (4 per cent.). In from ten to fifteen minutes about 80 per cent. of the kreatinin undergoes change, and after thirty minutes only a trace is present.

Another method was adopted in which the inhibitory power of a known amount of kreatinin on a weak sugar solution was determined. The kreatinin solution was then boiled with alkali for some time, and its inhibitory power again determined; the general results agreed with those obtained by means of the nitro-prusside reaction.

That some of the kreatinin remains as kreatin is likely from the fact that a solution boiled for fifteen minutes or so has a somewhat greater inhibitory effect than can be accounted for by the unchanged kreatinin present. This inhibitory power is, however, not nearly equivalent to what might be expected from a solution containing all the changed kreatinin in the form of kreatin, and the only available explanation is that a fair amount of the kreatinin has been destroyed while a certain amount is still represented by kreatin. Kreatin, when boiled with caustic alkali, is not nearly so quickly changed as kreatinin, and the first step here is probably the formation of sarkosin. Sarkosin possesses no apparent inhibitory effect on the reducing action of copper solutions by sugar, but it possesses fairly distinct reducing properties, and acts in a very short time. Thus, a urine boiled for some time with Fehling's solution contains mixtures of kreatinin, kreatin and sarkosin, and the longer it is boiled (within certain limits) the greater tendency there is for an increase in the latter ingredients and a decrease in kreatinin. Since kreatin is not very quickly acted on in alkaline solution, it is obvious that in boiling urine with Fehling's solution any kreatin derived from kreatinin will not tend to change very rapidly.

An attempt was made to determine the action of caustic alkali on urine by adding some sodium hydrate to urine, and after boiling for some time, testing the urine for kreatinin with sodium-nitro-prusside as described above. This was found to be quite impracticable,

for the action of boiling alkali and urine generates some substance (probably an acetone-like body derived from the carbo-hydrate material normally present) which, with sodium-nitro-prusside, gives the same reaction as kreatinin, and quite obscures the test for the detection of small amounts of this substance.

Another interesting point observed on boiling urine with caustic alkali may be mentioned here. After boiling for a very short time with alkali of moderate strength, the ordinary carbo-hydrate matter of urine seems to be completely destroyed. Urine having a reducing action equivalent to a solution containing .1 per cent. dextrose, was, after boiling for twenty seconds with 3 per cent. sodium hydrate, found to contain not a trace of carbohydrate substance as indicated by the most delicate tests for carbohydrate material. A .1 per cent. solution of dextrose in water containing from 2 to 3 per cent. sodium hydrate was found to be absolutely free from dextrose after boiling for from ten to fifteen seconds. In this way a urine can be rapidly freed from carbohydrate matter. While it is a well known fact that boiling or hot alkali destroys sugar, it would seem that the extreme facility with which this is accomplished is hardly appreciated.

THE ACTION OF THE ALKALINE PART OF FEHLING'S SOLUTION ON KREATIN

Kreatin, like kreatinin, reduces Fehling's solution only after prolonged boiling, and since some of the kreatinin of urine is changed into kreatin when boiled with Fehling's solution, some experiments were made to determine the effect on kreatin of boiling with an alkali. The inhibitory effect of the original kreatin solution was determined and compared with a sample which had been subjected to boiling with sodium hydrate for a definite time. In experiments a weak solution of sugar was used, and 1 c.c. of this mixed with 1 c.c. Fehling's solution; on heating a distinct reaction was obtained, which could be postponed and modified by the addition (before boiling) of a few drops of kreatin solution. In one such experiment it was found that 1 c.c. sugar solution + 1 c.c. Fehling's solution + three drops kreatin

solution, on boiling for ten seconds, gave, on standing for forty seconds, a distinct greenish opalescence. After boiling this kreatin solution (containing 4 per cent. sodium hydrate), tests were made in order to ascertain how many drops of the boiled kreatin solution gave a result as nearly as possible corresponding to the effect of three drops of the original solution. The following results were obtained.

Time original solution of kreatin boiled with alkali			No. of drops equivalent to three of original solution		Remarks
3 minutes	...		3	...	No appreciable change caused by boiling
6	„	...	4	...	Reaction with four drops some- what less distinct than with three original solution.
15	„	...	6	...	—
22	„	...	8	...	—

From the above it will be seen that kreatin is changed very much more slowly than kreatinin, when boiled with an alkali. After boiling for twenty-two to twenty-five minutes the solution still possesses fifty per cent. or more of its original inhibitory power, and since sarkosin has no inhibitory influence, we may assume that 50 per cent. of it is still present as kreatin. In boiling urine with Fehling's solution, any kreatin originally present as such, or formed from kreatinin, probably does not undergo any appreciable change in the short time during which the mixture is usually heated.

AN EXPLANATION OF THE REDUCING ACTION OF NORMAL URINE ON PROLONGED BOILING WITH FEHLING'S SOLUTION

As previously stated,¹ even normal urines give a very distinct reaction with Fehling's solution if boiled long enough. Pathological urines rich in dextrose, give a marked reaction even before the boiling point is reached, while the presence of a minute amount may necessitate boiling for some time in order to obtain any indication of the presence of sugar.

The chief reducing substances present in all normal urines

1. *Bio-Chemical Journal*, Vol. I, p. 111.

are uric acid, carbohydrate material (of which the larger part seems to be dextrose), and kreatinin. Uric acid and sugar, however, differ greatly from kreatinin in regard to the time required to cause reduction. Both substances, in the percentage in which they occur in normal urine, are capable of effecting reduction almost immediately on the boiling point being reached; in the process of reduction they undergo destruction.

Kreatinin, on the other hand, reduces very slowly indeed.

When a normal urine is boiled with Fehling's solution the uric acid and sugar present almost immediately reduce their equivalent amounts of the solution; no effect is perceived, owing to the fact that the reduced suboxide is held in solution by the kreatinin;¹ after boiling for a very short time, the full reducing effect of both uric acid and sugar is completed. On continued boiling the kreatinin gradually causes further reduction,² at the same time becoming gradually diminished in amount;³ part of it is probably converted into kreatin, while part is destroyed.

Ultimately a point is reached at which the suboxide reduced by the sugar and uric acid, added to that reduced by the kreatinin itself, is no longer capable of being held in solution by the amount of kreatinin and its derivatives actually present in the urine at that moment; at this point precipitation occurs.

Thus it will be seen that the reaction obtained from a normal urine is very similar in character to that obtained from a urine containing more than the normal amount of sugar, the chief differences being that the more sugar present the quicker the reaction occurs.

In the presence of great excess of sugar, the effect of kreatinin is, of course, quite obscured and of no practical importance. It will be seen that the influence of kreatinin on Fehling's solution is very marked and important, though somewhat different from that ascribed to it in the text books, where its action is generally considered in

1. See page 157.

2. Experiments with aqueous solutions of kreatinin in Fehling's solution show that kreatinin reduces very slowly, and in the percentage in which it is usually present in urine gives a precipitate only after prolonged boiling (say from four to six minutes).

3. See page 167.

relation to its direct reducing power. This direct action occurs but slowly, and is therefore very insignificant when Fehling's test is used in the ordinary way; its indirect inhibitory action, however, explains many points which are otherwise obscure and for which no definite explanation has been forthcoming.

CONCLUSIONS

1. Kreatinin is the substance in normal urine which most markedly interferes with Fehling's reaction in the presence of small amounts of sugar. Kreatin, if present, has a similar, though less marked, effect.

2. Experiment proves that kreatinin directly inhibits the effect of small amounts of sugar, when boiled with Fehling's solution, by holding the reduced suboxide in solution, and not indirectly by generating ammonia; the latter is not evolved in sufficient abundance to appreciably influence the reaction.

3. The yellow precipitate obtained in many urines containing sugar in pathological quantity is probably not the cuprous hydrate as generally stated, but the ordinary cuprous oxide, the difference in colour being dependent on the state of subdivision of the precipitate; in a yellow precipitate the particles are fine, while a red one is coarsely granular.

This difference is mainly caused by kreatinin. By varying the different amounts of sugar and kreatinin, all shades of colour from greenish-yellow to red may be obtained.

4. The colour changes observed on adding sodium-nitroprusside to an alkaline solution of either kreatinin or acetone are identical—a red colour changing to yellow, this change being accelerated by heat. Acetic acid subsequently added to the cold yellow solution gives, in the presence of kreatinin, a clear or light straw-coloured liquid, whereas acetone gives a distinct red or purplish tint.

In performing Salkowski's part of the test in which acetic acid and heat gives a blue colour, it is important not to heat the alkaline

mixture of nitro-prusside before adding the acetic acid, for sodium-nitro-prusside itself, when boiled in the presence of an alkali, is capable of giving a very distinct blue or bluish-green solution on the subsequent addition of acetic acid, apart from the presence of either kreatinin or acetone.

Acetone and kreatinin behave in exactly the same way to Salkowski's test—after alkaline nitro-prusside of sodium, the addition of acetic acid gives on subsequent boiling a green or blue coloured fluid, from which a precipitate ultimately settles.

5. Kreatinin, when boiled with caustic alkali of half the strength in which it is present in Fehling's solution, quickly diminishes in amount; part of it is probably converted into kreatin while part is destroyed; after boiling a weak solution of kreatinin for from four to seven minutes only about 50 per cent. of it remains.

6. Kreatin resists the action of caustic alkali much longer than kreatinin.

7. Sugar is very easily acted on by caustic alkali, and in weak solutions can be absolutely destroyed by boiling with 2-4 per cent. NaOH for some seconds.

8. The influence of kreatinin, as described above, explains why normal urine reduces Fehling's solution after prolonged boiling, and clears up many reactions in which a precipitate appears after boiling for some time with Fehling's solution.