VIII. ON THE CAUSE AND SIGNIFICANCE OF AN ABNORMAL REACTION OBTAINED IN TESTING URINE FOR SUGAR WITH FEHLING'S SOLUTION.

BY W. CRAMER.

From the Physiology Department, University of Edinburgh.

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The investigations recorded here were carried out as the result of observations made on a urine obtained accidentally from the Infirmary. The urine was supposed to contain glucose and did, in fact, give the usual tests for glucose. Nylander's test was positive; with phenylhydrazine typical glucosazone crystals were obtained; it fermented and it showed a dextrorotation indicating a percentage of 1.5 % sugar calculated as glucose. But when Fehling's test was applied, a curious and striking abnormality was observed. On following the usual routine method of heating to boiling point, in two separate test tubes, equal quantities of urine and of half diluted Fehling's solution and pouring the two hot solutions together a sudden change took place at once: a deep scarlet-red transparent solution was obtained. If this solution was observed in transmitted light without further heating, it began to darken after about ten seconds, at first slightly, then more rapidly. At the same time the solution became less and less transparent and eventually after about a minute a very fine black precipitate appeared, which remained suspended in the fluid. The precipitate was so fine that on filtering it passed partly through the filter paper. If the mixture was allowed to stand for 24 hours, the precipitate became partly aggregated in soft slimy masses, which settled out at the bottom of the vessel. It could then be removed by filtration.

In studying the conditions under which the reaction took place it was noted that it was possible to prevent this abnormal reaction from taking place by simply diluting the urine with an equal volume of water. If Fehling's test was applied to such a diluted urine the typical result—a yellow or red precipitate—was obtained.

If Fehling's solution and the undiluted urine were mixed in the cold and then heated together to boiling point (instead of heating them separately and mixing them when hot) a red precipitate was formed at first, which on further heating gradually darkened. This darkening became less and less marked when the relative amount of Fehling's solution with which a given volume of urine was mixed, was increased. The opposite condition—using an excess of urine and relatively little Fehling's solution—produced again a darkening of the cuprous oxide which was formed at first. If the red cuprous oxide obtained by heating together an excess of Fehling's solution and relatively little urine was added to a further quantity of the urine, and the mixture heated, the red precipitate again showed a darkening. It is perhaps necessary to state that the darkening of the fluid is quite different from that due to the action of alkali on sugar and that it is not due to the caramelisation of the sugar.

On investigating the nature of the black precipitate, it was thought at first from its physical appearance to be of an organic nature, perhaps a combination of copper with an abnormal organic constituent of urine. It was found, however, that the precipitate was not of an organic nature, for on heating weighed quantities of the precipitate in a crucible, it showed an increase in weight and formed a black mass, different in appearance from the original black precipitate and identical in appearance with cupric oxide, which, in fact, it was, as it yielded copper by being heated in a current of hydrogen. On treating the original black precipitate with acids, it was found that it dissolved readily in nitric acid with the evolution of brown fumes and formed a clear green solution: it was not readily affected by hydrochloric acid or sulphuric acid. It behaves therefore in the same way as metallic copper and is, in fact, copper in an extremely fine state of division, finer even than the copper obtained by reducing a cupric salt by means of a watery solution of glucose as described below. If the filter paper which has been used for the filtration of the precipitate is allowed to dry in the air, it acquires the characteristic green colour of basic copper carbonate (*verdigris*).

The fluid from which the precipitate had separated out had in transmitted light sometimes a brown, sometimes a greenish-black appearance, while in reflected light it was black, thus suggesting that it represented a suspension of very fine particles of the material which furnished the black precipitate, in other words that it was a colloidal solution of copper. This was confirmed by the fact that hydrogen peroxide was decomposed when added to the warm colloidal solution.

The conclusion arrived at, that the black precipitate is finely divided metallic copper, is less surprising since I have found that Fehling's solution is readily reduced by glucose to metallic copper. It was thought, at first, that reduction to copper would take place only if cuprous oxide were present in colloidal solution, and that therefore the presence of such substances as creatinine, which are capable of holding cuprous oxide in solution, was necessary. But this is not the case.

If a hot concentrated solution of glucose, or solid glucose, is added in excess to boiling Fehling's solution, one notices as the first change the well-known formation of red or yellow cuprous oxide. If heating is now continued for one or two minutes, this red or yellow precipitate darkens and the whole mixture assumes a dark brown colour. If at that stage the flame is removed, so that boiling is interrupted, an evolution of gas will be seen to be proceeding. If boiling is continued for three minutes a reddish brown heavy precipitate is obtained which rapidly settles at the bottom of the vessel and which, when washed and dried in a steam bath, is finally obtained as a fine powder having the characteristic colour of copper. It dissolves in nitric acid with the evolution of brown fumes. This and the increase in weight obtained on heating show that the precipitate consists almost entirely of metallic copper admixed probably with a small amount of cuprous oxide, which has not been further reduced.

The increase of weight obtained on heating should be 25.1 per cent. if the precipitate were metallic copper and 11.2 per cent. if the precipitate were cuprous oxide. The following figures were obtained with three different preparations:

1.	0.4274 g. precipitate	gave 0.5202 g. CuO.	Increase in weight, 21.71 %.
2.	0.2479 g. " "	0.3056 g. CuO.	" " 23.3 %.
3.	0.2385 g. " "	0.2954 g. CuO.	" " 23.85 %.

The last preparation had been dried in a current of nitrogen, and heated in a current of oxygen. As a control the CuO obtained was reduced again by heating in a current of hydrogen.

0.2954 g. CuO gave 0.2362 g. Cu. Decrease in weight, 25.06 %.

The only difference which exists between the reduction produced by pure glucose solution and that brought about by this urine consists in the state of division. Since in the latter case one obtains at first a colloidal solution of cuprous oxide, which is then further reduced to a colloidal solution of copper, the black precipitate which one eventually obtains as the result of the "coagulation" of the colloidal copper solution represents a

much finer state of division of the metal than the typically copper-coloured precipitate obtained with glucose in watery solution.

The black precipitate obtained from the urine was, of course, not chemically pure metallic copper, as it was mixed with the phosphates which had separated out from the urine as the result of boiling it with the alkaline Fehling's solution. It was probably also contaminated with some organic material adherent to it. Owing to this fact the increase in weight obtained on heating weighed quantities of the dried precipitate varied considerably (from 10 % to 14 %) and was below the value calculated for pure copper (25.1 %), but in some cases above that calculated for cuprous oxide (11.2 %).

It is now easy to understand the abnormal reaction described at the outset of this paper. When the heated urine and Fehling's solution were mixed, the glucose present in the urine at once reduced the cupric oxide to cuprous oxide. This, however, remained in solution for reasons which will be discussed presently, and then produced the transparent scarlet-red solution that has been described. This colloidal solution was then further reduced by the glucose to colloidal copper, which at first formed a colloidal solution from which it was gradually precipitated to form the black slimy masses of finely divided copper.

The question now arises: why is this reduction to metallic copper by urine containing glucose so rare, as it evidently is, since it has never been described? The answer is to be found in the following facts. The urine had the colour of normal urine. On diluting it with an equal volume of water, it gave, as has already been stated, the typical reduction with the appearance of cuprous oxide. On inquiry at the ward it was found that the total volume of urine excreted by the patient was rather below than above the normal. Dr R. A. Fleming, under whose care the patient was, and to whom I am greatly indebted for his readiness to place the material at my disposal, has very kindly given me all the details concerning the clinical aspects of the case, from which the following statement has been abstracted.

The case was demonstrated by Dr Fleming at a meeting of the Edinburgh Medico-Chirurgical Society [1914].

"The patient was a young woman, age 22 years, weight 8 st. 3½ lbs. She complained of 'weakness and tiredness' from which she had suffered for three years. Six months before admission to the hospital her doctor discovered sugar in the urine, for which she was treated by being placed on a strict diabetic diet.

The patient was very neurotic and frequently complained of 'Globus Hystericus' with palpitations on any excitement. She also had a degree of pharyngeal anaesthesia common in hysterical subjects. Apart from the excretion of sugar in the urine no other symptoms of disease were found.

The urine varied in amount from 24 oz. up to 52 oz., with an average of about 30 oz. in the 24 hours. The specific gravity of the urine varied from 1.030 to 1.035. There was no albumin in the urine. The amount of sugar varied greatly, from 0.4 grains per oz. and about 15 grains per day, to 5 grains per oz. and about 150 grains per day. Dietetic measures did not markedly affect the amount of sugar excreted. Apart from these measures she was treated also at first with codeine, later with a tonic containing strychnine. It was noted that whenever the sugar diminished the feeling of 'tiredness' was less marked.

After three months in hospital the patient gained 8 lbs. in weight and went out, feeling much better, although the excretion of sugar persisted."

Briefly summarised, the case is not a typical case of diabetes mellitus, but one of a marked glycosuria, unaccompanied by polyuria. At the time when these investigations were made, the urine contained about 1.5 % of glucose (estimated by the polarimeter). We have therefore to deal with a urine of normal concentration, in so far as the normal urinary constituents are concerned, which contains glucose in such amounts that more sugar is present than necessary to reduce all the cupric oxide, provided that the usual method of carrying out the test is applied (equal amounts of urine and half diluted Fehling's solution). Now if glucose is added to normal urine in such amounts that the urine contains about 1.5 % of glucose, and Fehling's test is applied to this urine, a deep red, transparent solution is obtained, which represents a colloidal solution of cuprous oxide. The appearance of this red solution instead of a precipitate of cuprous oxide is due partly at any rate to the presence of creatinine, which, as MacLean has shown [1907], is capable of holding cuprous oxide in solution. Other substances, perhaps the colloid material of urine acting as a protective colloid, also contribute to this effect. If this red solution is further heated it darkens to a deep brown solution and deposits a reddish brown precipitate, so that it is possible to imitate to a certain extent the abnormal reduction described at the outset of this paper simply by using a normal urine containing glucose in a certain concentration.

The change of colour from red to black is, however, not so striking in the case of normal urine to which glucose has been added. A much better imitation of the reaction can be obtained with normal urine in the following way.

Add to half a test tube full of normal urine two drops of chloroform, shake vigorously and apply Fehling's test at once. One obtains then the red, transparent, solution, which darkens without further heating and eventually deposits a black precipitate. If to urine which has been agitated with chloroform, and then allowed to stand so that the chloroform has time to settle, Fehling's test is applied, only the red solution is obtained which does not blacken on further heating. If now to this red solution sugar is added, blackening occurs.

It is therefore clear that this "abnormal" reaction is due to the presence in sufficient quantity of a reducing substance in urine of a normal concentration. The amount of the reducing substance present must be in excess of the amount necessary to reduce all the cupric salt used in the test.

The clinical interest which attaches to this reaction appears to be that it indicates the presence of relatively large amounts of sugar in a urine of normal concentration; it indicates, in other words, a marked glycosuria unaccompanied by polyuria.

Some years ago I obtained from the Infirmary a urine giving exactly the same reaction. Unfortunately, the patient left the hospital before I had time to investigate the condition. But as both this former and the present case were obtained accidentally, when material was being collected for teaching purposes, it seems likely that these "abnormal" reactions may occur much more frequently than would appear from the complete absence in the literature of any similar observation.

Since watery solutions of glucose are capable of reducing cupric salts to metallic copper, it is to be expected that urine from a typical case of diabetes mellitus, where we have a glycosuria accompanied by a polyuria, will also give the reduction to metallic copper. This is, in fact, the case, especially if excessive dilution be avoided by using Fehling's solution undiluted, or if Fehling's solution is diluted with an equal volume of normal urine. If after the appearance of the yellow or red precipitate of cuprous oxide boiling is continued, a marked darkening of the precipitate and of the solution occurs, varying from a light brown to the dark reddish brown copper colour, characteristic of the precipitate obtained by boiling Fehling's solution with a watery solution of glucose. It must be noted, however, that this further reduction occurs only when boiling is continued, and is even then not nearly so striking as the reaction described at the outset of this paper. The variations in colour depend on the amount of glucose in the urine, on the proportion of urine and Fehling's solution, and on the concentration of the mixture both as regards

the ordinary urinary constituents and the glucose. The darkening of the brick-red cuprous oxide precipitate must have been frequently observed, but it appears to have been ascribed generally to the caramelisation of the sugar by the alkali of Fehling's solution. That this is not the case is evident from the fact that exactly the same reaction is obtained, as stated above, if chloroform instead of glucose is added to the urine.

The reaction obtained with urine containing chloroform is also of interest, because it demonstrates that this "abnormal" Fehling's reaction is due mainly to the cuprous oxide dissolving power of normal urine. For chloroform shaken up with water gives with Fehling's solution only the red precipitate of cuprous oxide, which does not become further reduced to copper even after prolonged boiling.

SUMMARY.

1. A reduction of cupric salts to metallic copper can be brought about by concentrated watery solutions of the reducing sugars. It takes place even more readily when the reducing sugar is present in a urine of normal concentration with regard to the normal urinary constituents, being facilitated by the fact that some of the constituents of normal urine are capable of holding cuprous oxide in solution.

2. Clinically this abnormal reaction with Fehling's solution is of interest, as such a reaction may be taken to indicate a marked glycosuria unaccompanied by polyuria, and not a typical diabetes mellitus.

REFERENCES.

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