XV. A NEW TEST FOR REDUCING SUGARS IN URINE.

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The test depends upon the reduction of mercuric oxide in a weakly alkaline solution to metallic mercury. The degree of alkalinity is an important factor, as the test becomes more sensitive but less specific the greater the alkalinity of the reagent. The reagent is prepared as follows: 0.4 g. mercuric oxide (red or yellow) and 6 g. potassium iodide are dissolved in 100 cc. water. This solution is weakly alkaline. The alkalinity must now be so adjusted that 10 cc. of the reagent are neutralised by 2.5 cc. of $n/10$ acid, using phenolphthalein as an indicator. This is done by titrating 10 cc. of the reagent with $n/10$ acid and, after the alkalinity of the reagent has thus been determined, adding the requisite amount of $n/10$ acid or alkali to the bulk of the reagent. The reagent is a clear, colourless solution which turns slightly yellow on heating and becomes colourless again on cooling. It must remain quite clear on boiling. The reagent thus prepared will be referred to in this paper as the "2.5 standard reagent" in order to indicate its alkalinity.

Potassium cyanide may be used as a solvent for mercuric oxide, but potassium iodide has been found to be preferable, and all the data given in this paper refer to the reagent prepared with potassium iodide.

With this reagent the test if applied to an aqueous solution of a sugar is carried out as follows: 3 cc. of the reagent are heated in a test-tube to boiling. The solution remains clear, but turns slightly yellow. 0.3 cc. of the sugar solution is now added and the mixture again heated to boiling. The test-tube is then removed from the flame. The mixture darkens and a black precipitate of finely divided mercury appears. At first this precipitate gives the appearance of a turbidity, especially if the amount of sugar present is small. On standing, the precipitate soon settles at the bottom of the test-tube.

The test is positive with sugars which give the other reduction tests such as lactose, maltose, xylose and arabinose. It is negative with cane sugar.

An indication of the sensitiveness of the test is given by the fact that a slight reduction can still be obtained with one milligram of glucose if an aqueous solution of glucose is used.

If the test is applied to normal urine in the way to be described below, a slight but distinct reduction is obtained comparable in degree to that obtained with aqueous solutions of glucose containing 0.1 per cent. to 0.2 per cent. of glucose.

The alkalinity of the reagent has been fixed at the standard given above for the following reasons. The greater the alkalinity of the reagent the greater is the degree of a reduction produced by a given amount of a reducing sugar, so that the reagent could be made more sensitive by increasing its alkalinity. If, however, the reagent is made strongly alkaline, it ceases to be specific for reducing sugars and chemically allied substances, and is reduced by other organic substances, such as creatinine. And since sugars have to be identified as a rule in fluids such as urine and serum containing other organic substances, the reduction of the reagent by substances other than reducing sugars and chemically allied substances must be excluded, if the test is to be an improvement on Fehling's test, where the reduction by substances such as creatinine and uric acid constitutes a serious fallacy, particularly if small amounts of sugar are being tested for.

The alkalinity of the reagent was fixed at the 2.5 standard because at that point the reduction produced by normal urine is very slight and corresponds in degree to that produced by aqueous solutions containing 0.1 per cent. to 0.2 per cent. of glucose. It is then certain that the reduction produced by normal urine is due only to the glucose which it contains and not to any other constituent. This was confirmed by testing the reagent with a number of organic substances, present normally, or likely to be present, in urine, such as urea, uric acid, urates, guanine, allantoin, creatinine, hippuric acid, taurine, bile salts. In amounts exceeding those possibly present in urine all these substances gave negative results.

When the alkalinity of the reagent is increased so that 10 cc. of the reagent are neutralised by 3 cc. n/10 acid ("3.0 standard reagent"), normal urine gives a reduction slightly greater than that produced by 0.1 per cent. to 0.2 per cent. solutions of glucose. It does not necessarily follow that this greater reduction is due to some normal urinary constituent other than glucose. For the 3.0 standard reagent shows no reduction when tested with the urinary constituents mentioned above in concentrations exceeding those found in normal urine. The observations recorded in a previous paper [Cramer, 1915] suggest the possibility that, just as in the case of cupric

oxide, urine presents conditions more favourable for the reduction of mercuric oxide by glucose than a pure aqueous solution. But in order to exclude possible fallacies the 2.5 standard reagent is recommended and not the slightly more sensitive 3.0 standard reagent.

If, on the other hand, the alkalinity is reduced so that 10 cc. of the reagent are neutralised by 2 cc. n/10 acid ("2.0 standard reagent"), normal urine gives practically negative results, and a distinctly positive result is obtained only if the urine contains as much as about 0.3 per cent. of glucose. An example of the differences obtained with the reagents of different degrees of alkalinity is given in Table I.

TABLE I.

Glucose-solution	"2.0 Standard"	"2.5 Standard"	"3.0 Standard"
0.1 %	hardly visible	very slight	slight
0.2 %	slight	slight	slight
0.5 %	distinct	marked	intense
1.0 %	intense	intense	intense
Urine containing glucose			
0.1 % (normal urine)	hardly visible	very slight	distinct
0.2 %	hardly visible	slight	distinct
0.3 %	distinct	distinct	intense
0.5 %	distinct	intense	intense

There is, of course, no difficulty in recognising the presence of large amounts of reducing sugars by this reagent whether the "2.0 standard," the "2.5 standard" or the "3.0 standard" is used. But the value of this reagent lies in the fact that it enables one to recognise readily very small quantities of sugar in urine, when Fehling's test gives only doubtful results, while Nylander's test is less sensitive and requires for the identification of small amounts prolonged boiling which is often troublesome. A comparison of

TABLE II.

Specimen of urine	Glucose %	Fehling	Nylander	"2.0 Standard"	"2.5 Standard"	"3.0 Standard"
M	0.07	negative (transparent green)	negative	very slight	distinct	distinct
C 1	0.2	doubtful (opaque greenish brown)	doubtful	hardly visible	distinct	distinct
C 2	not determined	probably positive (transparent brown)	distinctly positive	distinct	distinct	distinct

the different tests is given in Table II. The tests were made with three different specimens of urine from two normal individuals. The amount of glucose present was determined by Bertrand's method, using the modification devised by MacLean and Gardiner [1914] for small quantities of glucose.

As Table I shows, the test here described enables one to recognise not only the presence of small amounts of reducing sugars, but also slight differences in the amounts of sugar present, when these amounts are small. But in order to do so it is necessary to carry out the test in such a way that the conditions under which reduction is produced are constant and that the turbidity, which is a measure of the amount of reduction, is due entirely to the precipitated mercury and not to the phosphates, which are, of course, also precipitated when the urine is added to the hot alkaline reagent. The turbidity due to phosphates can be excluded simply by acidifying the mixture, after the test has been carried out, with acetic acid, which dissolves the phosphates. Since the addition of acetic acid stops the reduction which would otherwise proceed in the hot mixture, it is necessary to add the acid after a fixed interval, say 30 seconds. As further the precipitate of mercury is heavy and rapidly falls to the bottom of the test-tube, the degree of turbidity must be judged at once. In the table the degree of turbidity is described as "intense" when ordinary print cannot be read through the mixture. The terms "slight" and "distinct" are applied when print can be read with ease through the mixture. The term "marked" means that print can be read only with difficulty. This "print reading test" is a useful criterion of the degree of reduction produced by small quantities of sugar. As stated above it must be applied immediately after the addition of the acetic acid.

The test, then, as applied to urine, which must, of course, not have undergone ammoniacal fermentation, is simply as follows: 3 cc. of the 2.5 standard reagent are heated to boiling in a test-tube (of ordinary dimensions). The reagent must remain clear. 0.3 cc. of urine is added and the mixture again heated to boiling. The test-tube is removed from the flame and, after thirty seconds, the mixture is acidified with a few drops of acetic acid. The test-tube is at once held over ordinary print. If the urine is normal, a slight but distinct turbidity remains and print is clearly readable through the mixture.

It may be suggested that the "2.0 standard reagent" is more suitable for clinical use as it gives a negative result with normal urine. But there is no reason why the small amount of glucose present normally in urine should be deliberately ignored, if it can be readily recognised. Moreover, the differences in the degree of reduction given by urines containing a slight excess of glucose (0.2 per cent. to 0.5 per cent.) are better marked with the "2.5 standard reagent" than with the less sensitive "2.0 standard reagent."

The reduction of mercuric salts by sugar in alkaline solution has been used before by Knapp [1870] as a method for the quantitative estimation of sugar. His solution consists of a strongly alkaline solution of mercuric cyanide. The method has not, however, come into general use, at least not for biochemical work. For the solution is stated [Browne, 1912] to be reduced not only by sugars but also by creatinine, glycerol and a number of other organic substances other than sugars, and is therefore unsuitable for biochemical work either as a qualitative test or as a quantitative method. The same objection applies to the modifications of Knapp's solution by Sachsse and Bauer [quoted by Browne, 1912]. From the observations recorded in this paper it would appear that the non-specific nature of Knapp's solution is due mainly to its strong alkalinity. Indeed, if the reagent described in this paper is made very strongly alkaline by adding strong caustic soda, it is readily reduced even by glycerol. Observations are at present being carried out to determine whether the reaction described in this paper is suitable for the quantitative estimation of sugar in urine and other physiological fluids.

In conclusion the advantages of the test described in this paper in comparison to Fehling's test and Nylander's test as applied to urine may be stated. They are as follows:

1. The test is free from the fallacies inherent in Fehling's test as the result of the reducing action of uric acid and creatinine on Fehling's solution.
2. It is more easily carried out than Nylander's test, especially when small amounts of sugar are tested for.
3. With small quantities of sugar it is easier to judge roughly the amount of sugar present from the degree of turbidity obtained in this test than from the changes observed in Fehling's or Nylander's test. The test is in fact particularly suitable when the amounts of sugar present exceed the normal amount only slightly; that is to say in those conditions where Fehling's test gives ambiguous or doubtful results.
4. It is more sensitive than Fehling's or Nylander's test and allows a distinction to be made between the amount of sugar normally present in urine and that slightly exceeding the normal.

REFERENCES.

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