Simple Specific Test for Urine Glucose

Alfred H. Free, Ernest C. Adams, Mary Lou Kercher, Helen M. Free, and Marion H. Cook

PROCEDURES FOR THE DETECTION OF SUGAR IN URINE ARE AMONG THE OLDEST TESTS KNOWN IN CLINICAL CHEMISTRY. LEGENDS GOING BACK TO EARLIEST TIMES DESCRIBE THE ATTRACTION OF INSECTS TO URINE CONTAINING SUGAR. IN MANY INSTANCES IT IS DIFFICULT TO SEPARATE LEGEND FROM REALITY IN TRACING THE HISTORY OF TESTS FOR GLYCOUSURIA.

AMONG SUCH PIONEER AMERICAN CLINICAL CHEMISTS AS STANLEY BENEDICT, OTTO FOLIN, VICTOR MYERS, AND DONALD VAN SLYKE, THERE WAS GREAT INTEREST IN ESTABLISHING AND USING TESTS FOR THE DETECTION OF SUGAR IN THE URINE. FROM THE MANY TESTS DESCRIBED DURING THE EARLY PART OF THE TWENTIETH CENTURY, BENEDICT'S COPPER REDUCTION TEST EMERGED AS THE ONE OF GREATEST POPULARITY. DURING THE PAST 10 YEARS CLINITEST, WHICH IS A SELF-HEATING ALKALINE COPPER REDUCTION TEST, HAS RECEIVED WIDE ACCEPTANCE IN THIS COUNTRY AND IN MANY OTHER PARTS OF THE WORLD. THIS TEST WILL SUBSEQUENTLY BE REFERRED TO AS THE TABLET COPPER REDUCTION TEST.

THIS REPORT DESCRIBES A NEW, SIMPLE TEST FOR THE IDENTIFICATION OF GLUCOSE IN THE URINE. THE TEST DEPENDS ON THE ACTION OF AN ENZYME AND THUS BY-PASSES THE CLASSICAL PRINCIPLE OF ALKALINE REDUCTION OF METALLIC IONS. THE TEST IS CALLED CLINISTIX, AND WILL HEREAFTER BE REFERRED TO AS THE GLUCOSE OXIDASE TEST.

METHODS

THE REAGENTS INVOLVED IN THE GLUCOSE OXIDASE TEST ARE GLUCOSE OXIDASE, PEROXIDASE, AND ORHTOTOLIDINE. THEY ARE IMPREGNATED INTO A STRIP OF STIFF PAPER. IN ORDER TO CARRY OUT A TEST, THE STRIP IS MERELY DIPPED INTO THE
urine specimen. One minute later the strip is observed for the appearance of a blue color which indicates the presence of glucose.

The enzyme glucose oxidase was first identified by Muller (1) approximately 30 years ago. In the presence of oxygen the enzyme catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. In the absence of oxygen the reaction of glucose and glucose oxidase may utilize certain organic compounds as hydrogen acceptors. In the glucose oxidase test the hydrogen peroxide formed reacts with orthotolidine, the reaction being catalyzed by a second enzyme—peroxidase. This reaction results in the development of a deep blue color.

**EXPERIMENTAL**

The glucose oxidase test is very sensitive for the detection of glucose. Less than 0.01% glucose in water will give a positive reaction. Somewhat greater amounts of glucose in urine are required for a positive reaction, with 0.01% usually being negative and 0.1% glucose in urine giving a positive reaction. As with most other urine qualitative tests, the precise sensitivity depends somewhat on the nature of the sample. Table 1 shows typical quantities of glucose which need to be added to urine to get positive reactions with the tablet copper reduction test, Benedict's qualitative test, and the glucose oxidase test. The sensitivity of the enzyme test is such that urines with a small quantity of glucose either added or naturally occurring may give a positive reaction with the glucose oxidase test, a negative reaction with Benedict's and a negative reaction with the tablet copper reduction test. Whether the great sensitivity of the new enzyme test will be an advantage or a disadvantage has not been established. Many laboratories including our own (2) have established that the high sensitivity of Benedict's test is a disadvantage over a less sensitive test. However, Benedict's test is a test for total reducing substances rather than for glucose.

Glucose oxidase has been studied for the specificity of its reaction with glucose. Keilin and Hartree (3) reported that the enzyme had a high specificity for glucose but would react slowly with mannose, xylose,

<table>
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<tr>
<th>Test</th>
<th>Glucose concentration (%)</th>
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<tbody>
<tr>
<td>Tablet copper reduction test</td>
<td>0.15</td>
</tr>
<tr>
<td>Benedict's copper reduction test</td>
<td>0.08</td>
</tr>
<tr>
<td>Glucose oxidase test</td>
<td>0.05</td>
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</tbody>
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maltose, and galactose. The extreme sensitivity of the glucose oxidase test raises the question of whether it will react with concentrations of other sugars which may be encountered in the urine. It has been found that many commercial samples of sugars other than glucose give positive tests with the glucose oxidase test when tested in concentrated aqueous solutions. Purification of such sugars by fermentation with baker's yeast of any glucose present or by enzymatic removal of the glucose results in a nonreactive material. The sugars which have been established to be nonreactive with the glucose oxidase test at concentrations of 20% in urine are galactose, fructose, lactose, mannose, maltose, sucrose, xylose, D-ribose, D-arabinose, L-arabinose, and L-xylulose.

Glucose oxidase catalyzes the oxidation of β-D-glucose but has no effect on the oxidation of α-D-glucose. A freshly prepared solution of crystalline glucose contains only the alpha form and is essentially nonreactive with the glucose oxidase test. However, in solution an equilibrium mixture between the alpha and beta forms is readily established and within a few minutes reactivity with the glucose oxidase test appears, demonstrating the presence of β-D-glucose. In blood and urine containing glucose the equilibrium mixture of alpha and beta glucose is present so there is no problem of delayed reactivity, since β-D-glucose is available for immediate reaction with the enzyme.

In the treatment of diabetes, some clinicians regulate insulin dosage on the basis of the amount of sugar in the urine. For this reason quantitation of the amount of sugar in the urine is important. There are several factors which influence the amount of color in an enzyme test such as the glucose oxidase test. In addition to the effect produced by the sugar concentration the color is influenced by pH, temperature, and the concentration of antagonistic substances such as ascorbic acid. Accordingly, it has not been possible to quantitate the amount of glucose in urine. It is quite easy to demonstrate that with different amounts of sugar added to a given urine different colors are obtained. However, the variability of the urine and the inability of average operators to recognize small color differences makes it impossible to do little more than differentiate between small and large amounts of glucose in urine.

RESULTS

Table 2 shows the number of positive tests with the glucose oxidase test, the tablet copper reduction test, and Benedict's qualitative test obtained on a series of random urines from 352 healthy subjects. It will be seen that many positive reactions (trace) were obtained with Benedict's
test but only 2 positive reactions were obtained with the glucose oxidase test and 3 positive reactions with the tablet copper reduction test. A series of tests were applied to the urines giving negative reactions with the glucose oxidase test and the tablet copper reduction test but trace Benedict's tests. These included paper chromatography, measurement of the amount of reducing substance in the urine with the Nelson-Somogyi procedure (4) before and after fermentation with baker's yeast, measurement of the amount of reducing substances with Nelson-Somogyi procedure in the urine before and after aeration with glucose oxidase, and attempts to form osazone crystals. The results of these tests indicated that the urines from healthy subjects which gave trace reactions with Benedict's test but negative reactions with the glucose oxidase test contained nonglucose reducing substances. Confidence in the results of such tests is achieved by reason of the fact that addition of glucose to negative urines in quantities of 0.1 per cent can readily be recognized by paper chromatography, by decrease in total Nelson-Somogyi reducing substances after yeast fermentation or after aeration with glucose oxidase, and by a positive glucose oxidase test reaction.

With some urines this quantity of glucose can be identified by forming osazone crystals, but with other urines osazone crystals have not been obtained with this small amount of glucose.

A large number of tests have been carried out over a period of more than a year on random hospital urine specimens using the glucose oxidase test and one or two of the standard copper reduction methods, the tablet copper reduction test, and Benedict's qualitative test. These urine samples were tested as received and also following the addition of glucose. Table 3 shows data on 2075 random urines from hospital patients tested with the glucose oxidase test and Benedict's qualitative test. It can be seen that 1423 urines were negative with both tests. Approximately one-fourth of all the urines (571 out of 2075) gave trace reactions with Benedict's test but only 19 of these urines also gave a positive reaction with the glucose oxidase test. As with urines from healthy subjects the application of confirmatory tests to urines giving a trace reaction with Benedict's and a negative reaction with the glucose oxidase test showed that the reducing substance in these samples was not glucose. Fourteen

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>Glucose oxidase test</td>
<td>2</td>
<td>350</td>
</tr>
<tr>
<td>Tablet copper reduction test</td>
<td>3</td>
<td>349</td>
</tr>
<tr>
<td>Benedict's qualitative</td>
<td>33</td>
<td>289</td>
</tr>
</tbody>
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Table 2. URINE SUGAR TESTS ON HEALTHY SUBJECTS
of the urine samples were 1+ or more with Benedict's test but were negative with the glucose oxidase test. Two of these urines contained over 0.3% ascorbic acid which was a sufficient quantity to account for the positive test. In addition, among these urines were samples containing galactose, lactose, and fructose. There were 36 urine samples that gave positive reactions with the glucose oxidase test and negative reactions with Benedict's test. In these cases the amount of glucose present was quite small and not sufficient to give a positive Benedict's test. In these urines glucose was demonstrated by fermentation with baker's yeast with subsequent disappearance of the positive glucose oxidase test reaction and decrease in total Nelson-Somogyi reducing substance.

**Evaluation**

In order to assess the performance of the glucose oxidase test, samples of urine with or without added glucose were tested as unknowns by trained and untrained operators. Each day a few urines which gave negative reactions with the tablet copper reduction test and the glucose oxidase test were chosen. To each urine small amounts of concentrated aqueous glucose solutions were added to give several concentrations of glucose ranging from 0.1% to 4%. The samples were coded and given as unknowns...
to from one to five operators. Each operator tested each sample with the glucose oxidase test and recorded his result. Some rotation of operators occurred on different days so that every unknown was not tested by each operator. A separate trained operator tested each sample with the tablet copper reduction test. Results of the comparison of the copper reduction with the enzyme test are shown in Table 4. It is evident that the two tests have comparable accuracy with negative urines. The 2 per cent error with the glucose oxidase test and 1% error with the tablet copper reduction test on the negative urines was due to the operator’s obtaining trace reactions on urines which had been classified as negative. With amounts of added glucose between 0.1% and 0.25% the tablet copper reduction test was positive 82 per cent of the time. This figure depends on the exact amount of glucose added as well as on the specific urine to which it is added. The glucose oxidase test was positive 97 per cent of the time with amounts of added glucose between 0.1% and 0.25%. This demonstrates the high sensitivity of the glucose oxidase test. With amounts of added glucose of from 0.35% to 0.85% the glucose oxidase test was positive in 99 per cent of the cases. The 1 per cent of samples giving negative results had extremely high concentrations of ascorbic acid.

DISCUSSION

From the results of these studies it is apparent that the glucose oxidase test is a simple, accurate, specific, sensitive test for urine glucose. It has potential usefulness in diabetes detection screening programs, in routine urinalyses in hospitals and physicians’ offices, in the differential diagnosis of diabetic coma from insulin shock or other coma, in regular urine testing on known diabetics by both patients and physician, and in the differentiation of glucosuria from other melituriass.

SUMMARY

A simple, specific, sensitive, and speedy test for glucose in urine has been described. This enzymatic test based on the activity of glucose oxidase and peroxidase is called Clinistix. Data are presented to show that the test has a high accuracy with both positive and negative specimens.

REFERENCES