COMMERCIAL GLUCOSE OXIDASE PREPARATIONS FOR THE DETECTION OF GLUCOSE IN URINE

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ALTHOUGH enzymes which oxidize glucose are abundant in nature and have been known for many years, until recently, no application of their activity has been made in clinical biochemistry. The first enzymatic preparation with specific glucose oxidase activity was described by Müller[1] in 1928. Subsequent workers have isolated other preparations. The best known of these enzyme preparations is *notatin*, which Coulthard and associates[2] isolated from *Penicillium notatum* and described in detail in 1945. These enzymes are flavoproteins, and they catalyze the aerobic oxidation of glucose to gluconic acid with the production of hydrogen peroxide.

Recently there have appeared on the market two commercially prepared glucose oxidase reagents, Clinistix* and Tes-Tape,** which are designed to detect the presence of glucose in urine by color changes of reagent-treated paper. Each of these preparations is specific for glucose and no heating is required to bring about the reaction. This fresh approach to the detection of glucose after more than a century of exploitation of copper reduction by glucose seemed to warrant clinical trial of these two new preparations. Our report presents the results of a clinical trial, and evaluates both the new preparations and the conventional solution with respect to sensitivity, specificity, convenience, and economy.

Materials and Methods

The standard Benedict's technic used in our laboratory involves the use of Benedict's solution prepared from precompounded Benedict's reagent†. Five milliliters of reagent and 5 drops of urine are mixed and boiled for 5 minutes in a water bath. At the end of that time, readings are taken and are expressed in the usual semiquantitative manner as negative, or from (+) to (++++). Determinations of urinary sugar on a specimen showing (+++) reactions had a reducing sugar content of approximately 1 per cent as measured by the quantitative Benedict's procedure. This suggests that the method as we do it compares well with the manner in which it is carried out elsewhere.

Clinistix reagent strips consist of 2½-inch strips of heavy filter paper mounted like matches in a paper folder. Only the ends of the matchlike strips

Clinistix, reagent strips: Ames Company, Inc., Elkhart, Indiana.
**Tes-Tape, urine sugar test tape: Eli Lilly & Company, Indianapolis, Indiana.*
†*Purchased from the Paragon Test Laboratory, Orange, New Jersey.*

are impregnated with active material. To test urine for glucose, a strip is torn from the folder, the impregnated end of the strip is dipped into the urine specimen and is allowed to develop color. The reaction is not quantitative because in different specimens of urine many factors, including acidity, alkalinity, temperature, and amount of inhibitory substances, affect reactions of enzymes. The minimal concentration of glucose detectable by Clinistix ranges from as little as 0.01 per cent to 0.1 per cent, depending upon the amount of inhibitory substances present in the individual urine specimen. Thus, the color change is not proportional to the concentration of glucose in the urine.

Tes-Tape is a special paper impregnated with the enzymes, glucose oxidase and a horse radish peroxidase, in addition to an oxidizable substrate, ortho-tolidine. In the presence of glucose and moisture, the glucose oxidase reacts with the hydrogen peroxide and ortho-tolidine to develop a color in 60 seconds ranging from light green to blue-black, depending upon the concentration of glucose present. Tes-Tape is dispensed as a long roll of paper in a plastic dispenser similar to that used with many pH papers. A color scale is included which shows the different colors to be expected with increasing concentrations of urinary glucose. However, Tes-Tape does not distinguish in the important

Table 1. — *Results of 1340 parallel determinations using Clinistix and Benedict's reagent*

Type of test and result	No. of specimens
Both tests negative	1264
Both tests positive	56
Only Clinistix positive	20
Only Benedict's positive	0
Total	1340

Table 2. — *Results of 1000 parallel determinations using Benedict's reagent, Clinistix, and Tes-Tape*

Type of test and result	No. of specimens
All tests negative	945
All tests positive	38
Only Benedict's positive	0
Only Clinistix positive	1
Only Tes-Tape positive	4
Both papers positive, Benedict's negative	12
Total	1000

range between ½ per cent (+++) and 2 per cent or more (++++) of urinary glucose.

The first group of 1340 routine specimens of urine was subjected both to the Benedict's test and to the Clinistix test. The results of these tests are shown in Table 1. A short time after the completion of this series, Tes-Tape became available to us, and Table 2 shows the results of tests of 1000 routine specimens of urine using Clinistix, Tes-Tape, and Benedict's solution.

Comparison of Tests

Sensitivity. From these data, it is apparent that the sensitivity of the test papers is greater than that of our standard Benedict's test. Review of the charts of 15 patients from whom specimens were collected which gave positive oxidase and negative Benedict's tests indicated that the most common diagnosis was diabetes mellitus (10 patients). Other diagnoses included anxiety and nervous tension states (3 patients); back strain, arteriosclerotic cardiac disease, malignant glioma (1 patient each). Fasting blood sugar determinations on specimens from the five nondiabetic patients ranged from 65 to 85 mg. per hundred milliliters.

Specificity. The oxidase tests have the advantage of distinguishing glucose from other reducing substances in the urine, but no specimens are included in the present series in which a nonglucose-reducing sugar was present. A specimen in which this question was raised was sent to our chemistry laboratory and was submitted to the oxidase preparations to determine the nature of the reducing substance present. In this instance, both of the oxidase tests suggested that the reducing sugar was glucose. The oxidase in each of the preparations is specific, and negative tests were obtained with specimens of urine to which had been added lactose, fructose, zylose, or mannitol. A wide variety of drugs including acetylsalicylic acid, d-amphetamine, barbiturates, sulfonamides, quaternary ammonium compounds, and hexylresorcinol did not affect the reaction.

To compare further the specificity of the three tests, two normal persons were given 50 gm. of glucose by mouth, which is half the usual dose administered for the glucose tolerance test. In the one individual no glucose was found by the copper reduction or oxidase tests. However, for the other subject the glucose oxidase tests were positive at 15 minutes and at one and at two hours, although the Benedict's tests had been negative. Attempts to produce alimentary glycosuria, as measured by the oxidase paper tests, in this person by having him ingest high-carbohydrate food such as candy, failed even when the estimated amount of sugar eaten surpassed the 50 gm. Five patients submitted to glucose tolerance tests were studied by comparing the results of simultaneous tests of urinary excretion of glucose and the blood sugar levels. Among the urine specimens examined were three specimens that were negative to our standard Benedict's test and positive to the paper glucose oxidase tests. Determinations of blood sugar made at the time these specimens were collected ranged from 41 to 241 mg. per hundred milliliters.

Convenience and Economy. Under the conditions of the testing in our laboratory, the impregnated-paper preparations were not as convenient to use as the conventional qualitative Benedict's procedure. The manufacturers have designed the tests not particularly for use in the hospital laboratory but rather for small-scale use by the office technician or by the diabetic patient himself. In each paper method it is necessary to tear off the paper strips and to keep those individual bits of paper in order. We believe that this procedure is more complicated than the Benedict's test as it is performed in our laboratory. The three methods are about equally time consuming and, in view of their higher purchase price, it appears that each of the newer procedures would cost the hospital laboratory more per test than the copper-reduction method.

Of the two new products Clinistix was easier to use, while Tes-Tape was easier to read. The matchlike Clinistix papers were easy to dip into the urine specimens; whereas the Tes-Tape papers had a tendency to curl, making it necessary on occasion to use forceps for dipping the reagent paper into the urine. However, Tes-Tape is almost as quantitatively exact as is our Benedict's procedure, and it was made more convenient to use by first cutting the entire roll into strips and storing the small pieces of paper in a wide-mouthed glass-stoppered jar.

Summary

Clinical trial of two commercial glucose oxidase preparations shows them to be more sensitive in the detection of glucose in the urine than the conventional Benedict's test. The glucose oxidase preparations are not as convenient as the Benedict's test for large-scale testing; but, if their high sensitivity is taken into consideration, they are excellent products for use in the office laboratory, at the bedside, or by the diabetic patient himself. These reagents also are useful as reference tests in the determination of the nature of copper-reducing, non-glucose substances in urine because they are highly specific for glucose.

References

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