# Enzymatic Tests for Glucosuria

*John J. Moran, M.D., Paul L. Lewis, M.D.,*
*John G. Reinhold, Ph.D., and F. D. W. Lukens, M.D.,*
*Philadelphia*

A simple enzymatic test for glucose in urine was recently described independently by Keston,[1] Comeo[2] and by Free.[3] These tests employ paper strips impregnated with glucose oxidase, horse-radish peroxidase and O-tolidine as an indicator. The simplified mechanism of the reaction is as follows:

$$\text{glucose} + \text{glucose oxidase} \rightarrow \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{O-tolidine} \xrightarrow{\text{(peroxidase)}} \text{blue pigment}$$

A urine specimen may be tested for glucose by dipping the impregnated paper strip into the specimen. The strip is then exposed to air for one minute. The presence of glucose is shown by the development of a blue or green color.

It has been known for many years that reagents commonly used to test for glucose in urine react with reducing substances other than glucose. Thus an erroneous diagnosis of diabetes mellitus may be made because of the lack of specificity of the tests. Some of the substances which cause falsely positive reactions are fructose, galactose, arabinose, lactose, maltose, ribose, xylose, uric acid, glucuronic acid, homogentisic acid, salicylates, terramycin,[4] penicillin,[5] thiamine chloride,[6] para-aminosalicylic acid,[7] and streptomycin.[8] Proof that a reducing substance in a sample of urine is glucose may require considerable time and effort. The method of using glucose oxidase appears to be an improved test because of its specificity and simplicity, but insufficient data are available to establish its reliability and relationship to tests now in use. The present study was made to obtain such data.

## METHODS

Two commercial preparations utilizing glucose oxidase and a suitable indicator impregnated on paper were studied. Oxidase A was Clinistix made by Ames Company, Inc. and oxidase B was Tes-Tape made by Eli Lilly and Company.

The test strip was immersed in the urine specimen just long enough to wet the paper and was exposed to air for one minute. The development of a blue color in oxidase A or a green color in oxidase B was accepted as evidence of the presence of glucose.

Although product A was not recommended for quantitation, we found that the intensity of the blue color varied with the concentration of glucose. For this reason the degree of color change of the test strip was recorded as faint blue, light blue, or blue.

Product B was stated by the manufacturer to be suitable as a semiquantitative test for urine glucose. The results were recorded as one to four plus according to the color scale supplied by the manufacturer.

In some of the experiments to be described, the color intensities were compared with the results of a semiquantitative copper reduction test. Such tests were done by means of a self-boiling alkaline copper reagent for reducing sugars (Clinitest, Ames Company, Inc.).

In a smaller group of urine specimens, the results of the enzyme tests were compared with those of quantitative measurements of reducing substances. The method employed was the Nelson-Somogyi blood sugar method[9] adapted to urinalysis by our laboratory.

## TESTS FOR SPECIFICITY

Mannose, lactose, fructose, xylose, glucuronic acid and arabinose gave no color changes with the oxidase method either in aqueous solution or in urine, but reduced the copper reagent. Sucrose was nonreactive by both methods. Only glucose and galactose reacted with both the oxidase and copper reduction methods. The weak reaction of the latter with glucose oxidase was explained by the presence of glucose as an impurity, since the galactose solution did not react with glucose oxidase after treatment with a washed suspension of baker's yeast.

Albuminuria and hematuria may cause falsely positive tests for sugars in urine when copper reduction methods are used. Therefore the question arose whether the glu-

From the William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia.

cose oxidase test would be so affected. In a series of 223 urine specimens selected at random and showing negative tests for protein, 217 showed no evidence of glucose while six gave positive reactions when tested with oxidase A. The incidence was nearly identical in a similar series of 221 specimens in which albumin was present, obtained from the same population of patients as the preceding. Of these 214 were negative for glucose while seven were positive. If albumin affected the glucose oxidase test, it would appear that a greater frequency of positive tests should have occurred in the latter group. Since there was no difference it would appear that albuminuria has no effect.

Seven urine specimens showing gross hematuria gave negative tests for glucose with the oxidase and copper reduction methods. Thus hematuria does not interfere with the glucose oxidase test.

## COMPARISON OF THE ENZYMATIC AND COPPER REDUCTION METHODS FOR DETECTION OF GLUCOSURIA

A total of 2,002 urine specimens were tested by means of glucose oxidase strips and the semiquantitative copper reduction method. Of this total, 1,493 were obtained from diabetic outpatients and 509 from unselected hospitalized patients.

In the diabetic group (figure 1) both oxidase tests and copper reduction tests were negative in 56 per cent of specimens with oxidase A and 49 per cent with oxidase B. Both oxidase and the copper reduction tests were positive in 32 per cent with oxidase A and 35 per cent with oxidase B. Thus the results agreed in a total of 88 per cent with oxidase A and 84 per cent with oxidase B.

In the urine specimens from hospitalized patients (figure 2), both oxidase tests and copper reduction were negative in 90 per cent of specimens with oxidase A and 96 per cent with oxidase B. Both oxidase and copper reduction tests were positive in 5.1 per cent of specimens tested with oxidase A and 1.5 per cent with oxidase B. The agreement between enzymatic and copper reduction tests was somewhat higher than in the diabetic group, being 95.1 per cent with oxidase A and 97.5 per cent with oxidase B.

Disagreement was most frequent in the diabetic patients with oxidase reactions and negative copper reductions. This occurred in 9.1 per cent of specimens tested with oxidase A and 12 per cent of those tested with oxidase B. The corresponding figures for the hospitalized group were 2.8 per cent with oxidase

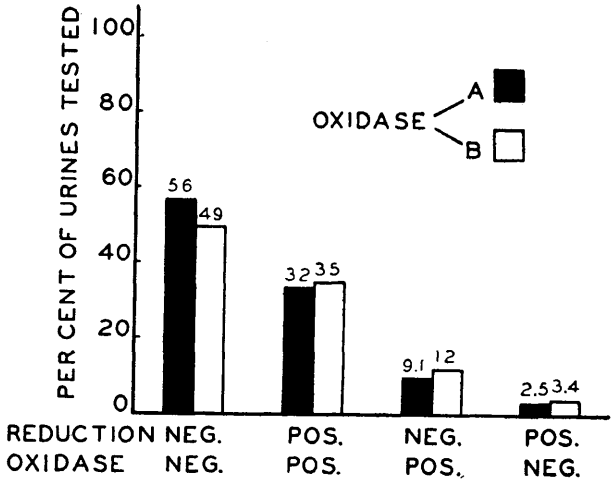


FIG. 1. Copper reduction method for sugar in urine compared with glucose oxidase test strips. One thousand, four hundred and ninety-three urines were tested with oxidase A and 655 with oxidase B. All were from diabetic outpatients.

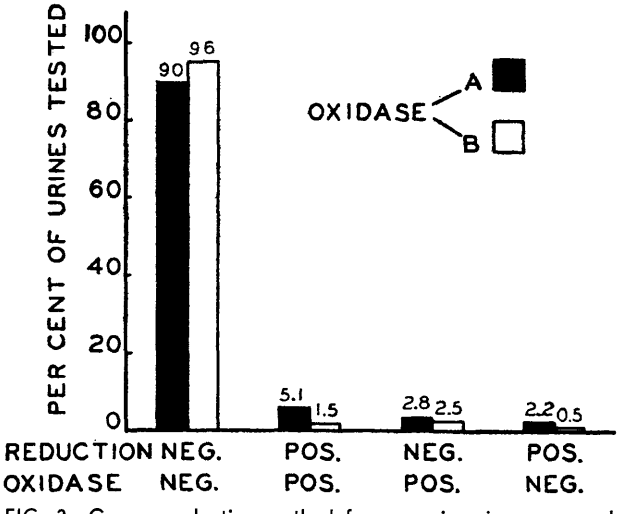


FIG. 2. Copper reduction method for sugar in urine compared with glucose oxidase test strips. Five hundred and nine urines were tested with oxidase A and 198 with oxidase B. All were selected at random from hospitalized patients.

A and 2.5 per cent with oxidase B. The reason for the occurrence of a positive oxidase test and a negative copper reduction test is the greater sensitivity of the oxidase reaction.

In the diabetic group the oxidase test was negative and the copper reduction test was positive in 2.5 per cent of urine specimens tested with oxidase A and 3.4 per cent tested with oxidase B. In the group of urine specimens from the hospitalized patients the corresponding figures were 2.2 per cent and 0.5 per cent with oxidase A and B respectively. Reducing substances other than glucose would account for these results.

## EVALUATION OF GLUCOSE OXIDASE STRIP PREPARATIONS FOR MEASUREMENT OF GLUCOSE CONCENTRATION

Figures 3 and 4 show comparisons of the intensity of the color reaction of the glucose oxidase test strips with the quantitative measurement of sugar in urine. One hundred and seventeen urines were tested with oxidase A and 107 with oxidase B. These specimens were obtained from diabetic outpatients.

Although there is a general correlation between the glucose concentration in urine and the intensity of the glucose oxidase tests, the gradations in color do not sharply distinguish between the different concentrations of glucose. A number of weakly positive tests with both enzyme preparations were associated with urine glucose concentrations of less than 0.1 per cent, and a few scattered specimens gave strongly positive tests with glucose concentrations in this range. A preponderance of the more strongly positive tests occurred in the urine containing more than 0.4 per cent of glucose. The absence of any negative reaction in urines having more than 0.2 per cent glucose indicates that such a response excludes clinically significant glucosuria.

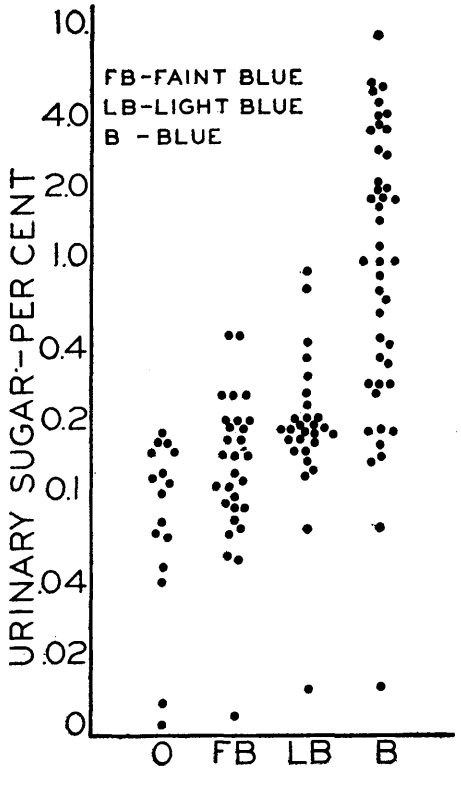


FIG. 3. This relates the tests with oxidase A to the quantitative determination of reducing substances from diabetic outpatients. Concentrations of glucose in urine are shown on the logarithmic scale. The lower cycle shows concentrations of reducing substance up to 0.1 per cent, the center cycle from 0.1 to 1.0 per cent, and the upper cycle from 1.0 to 10.0 per cent.

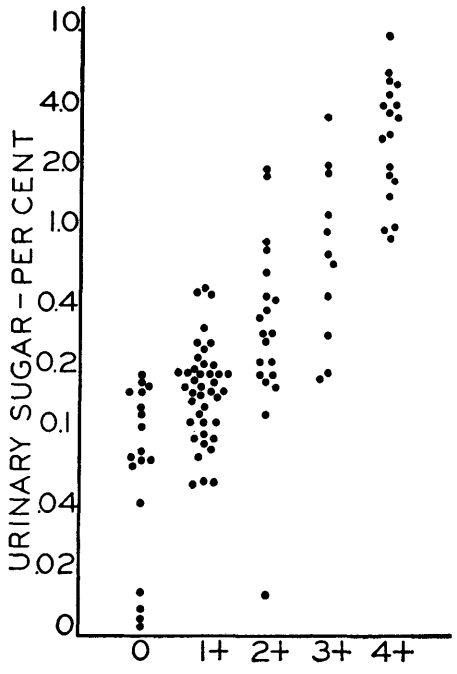


FIG. 4. This relates the tests with oxidase B to the quantitative determination of reducing substance in 107 urines from diabetic outpatients. See figure 3 for explanation.

The wide range of glucose concentrations associated with the production of a given color intensity shows that glucose oxidase test papers have distinctly limited usefulness for the semiquantitative measurement of glucose concentrations in urine. One exception in which the grading of the color intensity was helpful is for detecting glucose concentrations of 1 per cent or greater in the urine of known diabetics. All urine specimens containing 1 per cent or more of glucose gave a blue reaction with oxidase A. The detection of urine sugar of 1 per cent or higher is considered by the Diabetes Clinic of this hospital to be important in the control of diabetes mellitus. A less intense color reaction, therefore, could be accepted as evidence that the specimen contained less than 1 per cent glucose. However, it should be noted that a blue reaction often occurred when less than 1 per cent glucose was present.

Oxidase B may be used in a similar way since reactions of 2+ and higher were found in all urine specimens in which the glucose concentration exceeded 1 per cent.

Unless the user takes into account the much greater sensitivity of these glucose oxidase tests as compared with the older methods, misleading conclusions may be

drawn concerning positive tests. The color reactions both of oxidase B and of the copper reduction methods are graded as to one to four plus. However, on the label the approximate range of a one-plus reading with oxidase B is "about 0.1 per cent," while with a copper reduction one plus represents 0.5 to 1.0 per cent. This difference in range applies throughout the scale, except for the four-plus reading (above 2 per cent). Although the range is clearly stated by the manufacturer of oxidase B, confusion could result, particularly in the minds of the patients.

## APPLICATION OF THE ENZYMATIC TESTS TO URINALYSIS

Glucose oxidase used in the form of test papers for the detection of glucosuria has great advantages over older methods in simplicity and specificity. It is well adapted to rapid screening of routine urines for glucose. When so used, the finding of a positive test should be supplemented by a quantitative measurement by a dependable method to establish the amount of sugar if this information is required.

Glucose oxidase offers a most convenient and valuable means of ruling out falsely positive copper reduction tests due to reducing substances other than glucose. For this purpose it is superior to the older technics depending upon fermentation with yeast, formation of osazones, or paper chromatography.

The oxidase method has been in use in the Diabetes Clinic of this hospital for six months. For the first few weeks, the results were compared with those of the copper reduction method. It soon became obvious that although there might be differences in single samples of urine, the total number of sugar-free samples per clinic day was the same by both methods. This has led to the conclusion that the greater sensitivity of the oxidase method does not interfere with its practical use in observing and maintaining freedom from glucosuria provided the light blue and faint blue color reactions are considered negative.

It appears that glucose oxidase test strips will be useful for two purposes: (a) for the diagnosis of diabetes in which the ease of the method will facilitate screening in doctor's office or health center and (b) in the control of diabetes, especially the mild (i.e. stable) form, in which freedom from glycosuria can be observed with this test. The greatest difficulty in introducing this test in our clinic was the campaign of education needed to eliminate the traditional one to four plus recording of results. Unpublished data indicate that these are too erratic to justify the attention they have received in the past.

## SUMMARY

The results of 2,002 oxidase tests of urine glucose were compared with those of copper reduction methods. Of these, 1,493 were from diabetic outpatients and 509 were random urine samples from hospitalized patients. The results of the oxidase and copper reduction methods agreed in 85 to 88 per cent of the diabetic group and in 95 to 97 per cent of the hospital group. The discrepancies in the diabetic group were mainly a result of the greater sensitivity of the oxidase tests.

Glucose oxidase papers did not accurately measure concentrations of glucose in urine, although some information of clinical value, especially concerning the occurrence of higher concentrations, i.e., 1 per cent of glucose or greater, could be obtained by grading the intensity of the color formed.

## SUMMARIO IN INTERLINGUA

*Probas Enzymatic Pro Glucosuria*

Le resultados de 2.002 probas a oxydase urinari esseva comparate con le resultados de methodos a reduction per cupro. Le serie total del specimens includeva 1.493 ab diabetic patientes visitante; 509 esseva specimens urinari ab non seligite patientes hospitalisate. Le resultatos del methodos a oxydase e a reduction per cupro esseva conforme in 85 a 88 pro cento del gruppo diabetic e in 95 a 97 pro cento del gruppo hospitalisate. Le discrepantias in le gruppo diabetic reflecte principalmente un plus alte grado de sensibilitate in le proba a oxydase.

Papiros a oxydase de glucosa non mesurava accuratemente le concentrationes de glucosa in le urina, sed per graduar la intensitate del resultante color il esseva possibile obtener certe informationes de valor clinic, specialmente in re le occurrentia de plus alte concentrationes de glucosa—i.e., concentrationes de 1 pro cento o plus.

## ACKNOWLEDGMENT

This study was supported by a grant from Ames Company, Inc., Elkhart, Indiana.

## REFERENCES

1 Keston, A.: Specific colorimetric enzymatic analytical reagents for glucose. Abstracts Am. Chem. Soc. 129th Meeting, April 1956, Dallas, Texas.

2 Comeo, J. P.: A semiquantitative specific test paper for glucose in urine. Abstracts Am. Chem. Soc. 129th Meeting, April 1956, Dallas, Texas.

3 Free, A. H., Adams, E. C., Kerchner, M. L.: A simple specific test for urine glucose. Abstracts, International Congress on Clinical Chemistry, September 1956, New York, New York.

4 Lippman, R. W.: Effect of antibiotic agents on the test for protein and reducing sugar in urine. Am. J. Clin. Path. 22:1186-88, 1952.

[5] Whipple, R. L., and Bloom, W. L.: The occurrence of false positive tests for albumin and glucose in urine during the course of massive penicillin therapy. J. Lab. & Clin. Med. 36:635-39, 1950.

[6] Hart, R. S., and Wise, L. E.: The possibility of faulty diagnosis in patients taking thiamin chloride. J.A.M.A. 112:423, 1939.

[7] Zarafonetis, C. J. D., and Chandler, J. P.: Reducing substance in urine of patients treated with para-aminobenzoic acid. J. Lab. & Clin. Med. 37:425-30, 1951.

[8] Neuberg, H. W.: Streptomycin as a cause of false-positive Benedict's reaction for glycosuria. Am. J. Clin. Path. 24:245-46, 1954.

[9] Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375-80, 1944.

## *Essential Fatty Acids*

Although the exact and optimal requirements for fat in the diet have not been established precisely, it has been demonstrated that in the complete absence of dietary fat, normal growth and well-being are not possible. In 1929, Burr and Burr reported that rats on fat-free diets failed to grow normally and developed a scaliness of the skin and tail. Kidney lesions were also present; in the female ovulation was irregular and male rats on a fat-free diet could not be induced to mate. The syndrome which develops could be completely alleviated and the animals restored to normal health by the administration of linoleic acid ($C_{17}H_{31}COOH$). No other fatty acid tested was curative for the fat-deficiency, and high-grade butter fat was also ineffective, thus supporting the view that the fat-soluble vitamins were not concerned with the development of the deficiency condition. A few years later the same laboratory reported that linolenic acid ($C_{17}H_{29}COOH$) was also effective in alleviating the fat-deficiency manifestations. It was suggested that the capacity of the animal organism to synthesize certain unsaturated fatty acids is limited and that these fatty acids, e.g., linoleic and linolenic acids, therefore, may be considered to be essential fatty acids in that they must be supplied in the diet.

The results of Burr and Burr were confirmed in several laboratories and the concept of essential fatty acids extended to include the $C_{20}$, four double bond fatty acid, arachidonic acid ($C_{19}H_{31}COOH$). Turpeinen and Smedley-MacLean and her associates demonstrated that arachidonic acid was effective in curing the fat-deficiency disease in rats and, indeed, that this fatty acid was superior to the other essential fatty acids in curative power. Arachidonic acid, administered as the methyl ester, was found to be more active than methyl linoleate when judged by weight increase of the experimental animals. The two esters were approximately of equal potency with respect to ability to cure skin lesions. Smedley-MacLean and her colleagues concluded that arachidonic acid is necessary for new tissue synthesis. In circumstances of diminished dietary intake, its concentration in the subcutaneous tissue is depleted as a result of supplying the rapidly growing parts.

A state of chronic essential fatty acid deficiency has been produced in adult mice without the outward symptoms of acute deficiency, except for growth inhibition. In these animals, stress conditions, such as injury, pregnancy and x-irradiation precipitated the animals into the acute deficiency state. Growth inhibition due to essential fatty acid deficiency is not modified by hypophyseal growth hormone injection. Symptoms of essential fatty acid deficiency have also been described in dogs. Puppies on a low-fat diet developed a generalized flaky desquamation with coarse dry hair at about three months of age. These skin changes were prevented by the inclusion of lard in the diet.

Dogs on a low fat diet (1 per cent of the calories as fat) showed skin lesions and a lowered content of conjugated unsaturated fatty acids in the serum and skin.

At the present time too little is known about the requirement of man for unsaturated fatty acids. Two infants maintained by vonGröer on a diet very low in fat grew quite well, but one developed a generalized eczema. Holt and his colleagues reported that one of three infants kept on a fat-free diet developed an eczema which was cured by feeding fat. A distinctly lower than normal blood content of both arachidonic and linoleic acid was found in infants with uncomplicated eczema. Some of the children showed remarkable clinical improvement after lard had been added to the diet. Similar findings were reported from another clinic. The data with human subjects are not as uniform as with experimental animals. This may be due to uncontrolled variables or species differences.

From the book *Diseases of Metabolism* edited by Garfield G. Duncan, M.D., published by W. B. Saunders Co., Philadelphia, 3rd ed., 1952, Section "Lipid Metabolism" by Abraham White, Ph.D., pp. 198-99.