Urine Glucose Testing by Glucose Oxidase Methods

A Critical Evaluation

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Highly sensitive enzymatic preparations for the determination of glucose in urine, although introduced only recently, have found wide acceptance by the medical profession in the clinical management of diabetic patients.[1] Utilizing the enzyme glucose oxidase combined with indicators, these new tests have the advantages of specificity for beta-glucose, rapidity of results, and simplicity of operation. There is need, however, for more critical evaluation of their interpretations.

Two commercially available products were studied in this investigation: Clinistix (Ames Company, Inc.) and Tes-Tape (Eli Lilly and Company). While fundamentally similar to Clinistix, the Tes-Tape uses a yellow-green color range to provide quantitative interpretations, while Clinistix turns blue with glucose concentrations as little as 0.01 to 0.1 per cent. The object was to ascertain the qualitative accuracy of both products and to appraise the quantitative value of Tes-Tape.

PROCEDURE

Aqueous glucose dilutions were prepared in the manner described in table 1 and then added to urines from nondiabetic patients (table 2) to provide a range of urine samples from negative through 5 per cent glucose. The urines used had first been found negative for glucose with Clinistix, Tes-Tape, and Clinitest.

A total of thirty-six urine samples were tested, each with ten dilutions, by thirty-six persons. These individuals, laboratory technologists, interns, and residents, each performed tests on three unknown urine specimens with ten coded dilutions. Readings were timed with a stop watch and read with Tes-Tape precisely one minute after immersion. All urines were then tested with Clinistix and again read at the end of one minute.

RESULTS

Results of this investigation are summarized in table 3. A total of 1,080 tests were performed with Tes-Tape,

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TABLE 1

Method of making dilutions

A 50 per cent glucose solution was prepared by dissolving 50 gm. of glucose in water and adjusting the volume to 100 ml. From this stock solution, a series of aqueous glucose dilutions were prepared as follows:

50%	= 5 ml.	50%	glucose	solution	+ 0 ml.	water
40%	= 4 ml.	"	"	"	+ 1 ml.	"
30%	= 3 ml.	"	"	"	+ 2 ml.	"
20%	= 2 ml.	"	"	"	+ 3 ml.	"
15%	= 1.5 ml.	"	"	"	+ 3.5 ml.	"
10%	= 1 ml.	"	"	"	+ 4 ml.	"
5%	= 1 ml.	"	"	"	+ 9 ml.	"
2.5%	= 2.5 ml.	5%	glucose	solution	+ 2.5 ml.	"
1%	= 1 ml.	"	"	"	+ 4 ml.	"
0	= 0 ml.	"	"	"	+ 5 ml.	"

TABLE 2

The final urine unknowns were then made by adding 0.4 ml. of the aqueous glucose dilution to 3.6 ml. of urine as shown below:

Per cent glucose in urine					
5	0.4 ml. of	50%	glucose	+ 3.6 ml.	urine
4	0.4 ml. of	40 "	"	+ 3.6 ml.	"
3	0.4 ml. of	30 "	"	+ 3.6 ml.	"
2	0.4 ml. of	20 "	"	+ 3.6 ml.	"
1½	0.4 ml. of	15 "	"	+ 3.6 ml.	"
1	0.4 ml. of	10 "	"	+ 3.6 ml.	"
½	0.4 ml. of	5 "	"	+ 3.6 ml.	"
¼	0.4 ml. of	2.5 "	"	+ 3.6 ml.	"
1/10	0.4 ml. of	1 "	"	+ 3.6 ml.	"
0	0.4 ml. of	water		+ 3.6 ml.	"

TABLE 3

Tabulation of results

Actual glucose concentrations		No. of tests	Interpretations by examiners: Per cent glucose with TesTape 0	0.1	0.25	0.50	2.0	Clinistix Neg.	Pos.
5.0	per cent	108	1	1	33	41	32	0	96
4.0	"	108	0	4	32	46	26	0	90
3.0	"	108	0	2	39	44	23	0	87
2.0	"	108	0	7	33	48	20	0	84
1.5	"	108	0	6	34	48	20	0	78
1.0	"	108	0	8	47	39	14	0	87
0.5	"	108	0	20	66	19	3	0	72
0.25	"	108	1	72	33	1	1	0	84
0.1	"	108	10	93	3	1	1	5	67
0	"	108	105	2	1	0	0	90	0

plus an additional 860 with Clinistix. From a qualitative standpoint, i.e., positive or negative for glucose, both tests showed a high degree of accuracy.

Quantitatively, Tes-Tape proved accurate in the lower ranges of glucose concentration (0 per cent to 0.1 per cent), but stronger concentrations of glucose were consistently underevaluated. Even when glucose dilutions of 5 per cent were tested, only thirty-two of the one hundred eight specimens were read as "4 plus."

DISCUSSION

While enzyme reactions are the basis of many tests used in clinical medicine, the fickleness of these procedures is well known to the clinical chemist and pathologist. These procedures are of considerable value in that they do not appear to be affected by other sugars and reducing substances which hamper the usual glucose tests, but as enzyme reactions they are subject to many variable factors. These tests undoubtedly have an optional *p*H and temperature, and may be accelerated by some organic and inorganic substances present in urine and inhibited by others. Heat, light, and humidity are all of importance. To obtain quantitative results from these reactions they must be rigorously controlled; such controlled conditions have not been advocated for these urine glucose tests. As a result, attempts to quantitate these procedures are likely to fail to provide an acceptable degree of accuracy.

Such a situation appears to exist with the product, Tes-Tape. It is obvious that in clinically significant ranges of glucosuria there is considerable inaccuracy, and that such inaccuracy may easily have dire consequences in the poorly controlled diabetic on the verge of acidosis. On the other hand, the extreme sensitivity of both of these tests to minute concentrations of glucose in the urine must be made very clear to the patient lest he overdose himself into insulin shock.

We feel that these preparations do have considerable value as qualitative tests for glucose. One of these products has been used in the urinalysis laboratory of Harper Hospital as a screening test for a period of six months with encouraging results. All positive results are then checked with one of the accepted quantitative methods. This technic has proved rapid, efficient, and inexpensive.

CONCLUSION

A survey in which thirty-six physicians and technologists interpreted 360 glucose dilutions in urine disclosed that while Tes-Tape and Clinistix are qualitatively accurate, the former gives misleading quantitative results with glucose concentrations over 0.1 per cent. Higher concentrations of glucose were consistently underevaluated, a situation which could prove dangerous in the poorly controlled diabetic. Both products appear to be useful as screening procedures in the clinical laboratory.

SUMMARIO IN INTERLINGUA

Tests de Glucosa Urinari per Methodos a Oxydase de Glucosa: Un Evalutation Critic

Trenta-sex medicos e technologos participava in iste studio per interpretar 360 dilutiones urinari de glucosa, demonstrante que Tes-Tape e Clinistix es qualitativemente accurate sed que Tes-Tape produce erronee resultatos quantitative quando le concentrations de glucosa excede 0,1 pro cento. Plus alte concentrationes de glucosa esseva uniformemente subestimate, un facto que poterea devenir periculose in patientes de diabete non firmemente dominate. Ambe le mentionate productos pare esser de valor in le segregation preliminar de specimens in le laboratorio clinic.

REFERENCES

1 King, J. W., and Hainline, A.: Commercial glucose oxidase preparations for the detection of glucose in urine. Cleveland Clin. Quart. *23*:212-15, July 1956.

Pentoses

In contrast to the hexoses, which are important energy materials, the 5-carbon atom sugars are much more important as part of the machinery of the body. Pentoses are incorporated in at least one vitamin (riboflavin), several tissue coenzymes (diphosphopyridine nucleotide, tri-phosphopyridine nucleotide, alloxazine adenine dinucleotide), and all the nucleoproteins. However, when pentoses as such are ingested, they are not utilized but are eliminated, more or less quantitatively, in the urine and feces. It is possible that the pentoses which are eaten in combined form as part of natural food constituents (riboflavin and the nucleotides, for example) do contribute to the pentose content of the tissues. It is known that the body is able to synthesize pentoses for itself — from glucose by way of glycuronic acid.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955, Chapter "The Role of Carbohydrates in the Diet" by Samuel Soskin, M.D., and R. Levine, M.D., p. 160.