The Use of Simple Enzymatic Tests for Glucose in Detection of Diabetes

Evaluation by Means of a Modified Glucose Tolerance Test

Frederick C. Goetz, M.D., and Peggy Rose Guggenheim, Minneapolis*

Paper strips impregnated with a glucose oxidase enzyme system are now well known in the detection of glucose in the urine. Whatever limitations they may have in the day-to-day management of known diabetes, their extreme simplicity suggests very great usefulness in the detection of unknown cases of diabetes.

However, two aspects of their use have caused some hesitation in this regard: 1. Since the enzyme tests are self-developing, the imperfectly informed lay person might make unwarranted and possibly harmful conclusions concerning his own condition without the benefit of professional medical advice. To be sure, in most detection campaigns final action in seeking medical advice is up to the individual lay person, regardless of the method of detection used. 2. The enzyme tests are very sensitive, detecting 100 mg. of glucose per 100 ml. or even lower concentrations, and there is a reasonable concern that an unusually large number of false positive tests may result. Undesirable effects of this sensitivity would include wasted effort on the part of the detection group and unnecessary alarm and anxiety on the part of some members of the public. It is also possible that either fading or late development of the color in the enzyme tests might affect the results unpredictably.

An opportunity to gain information regarding these questions was presented by the diabetes detection drive sponsored by the Twin Cities Diabetes Association of Minneapolis and St. Paul as part of National Diabetes Week, Nov. 11-17, 1956. A modified glucose tolerance test was used to interpret the results of the urine tests. Comparison was possible between the two enzyme tests and the Dreypak, a copper reduction method widely used in diabetes detection.[1]

The results indicate that some self-interpretation of the enzyme tests undoubtedly occurred, but that this probably did not affect materially the number of cases reported to the detection committee. The enzyme tests appeared to have the same over-all degrees of sensitivity and reliability as the Dreypak.

METHODS

Format of the Tests: Tes-Tape and Clinistix are paper strips impregnated with a glucose oxidase-orthotolodine system so that a blue color develops in the strip in the presence of glucose and oxygen.[2,3] They differ mainly in that Tes-Tape comes as thin yellow paper and Clinistix as rather thick white paper. Testing units supplied by Eli Lilly and Company consisted of a strip of Tes-Tape stapled to a foil-lined paper wrapper folded loosely over it. Clinistix testing units were supplied by Ames Company, Inc., and consisted of a strip of Clinistix enclosed in a tightly sealed foil envelope attached to a printed card. Both units provided space for recording identifying information and also for recording in simple fashion the results of the test as done at home—that is, whether or not a color developed in the strip. The Tes-Tape unit provided a simple color chart for matching.

The Dreypak was devised by Dr. Norman Drey for the St. Louis Diabetes Association. It consists of a strip of filter paper impregnated with sodium fluoride as a preservative and attached to a broader strip of plastic and cardboard. The filter paper is dipped into a urine specimen and allowed to dry. At the detection center the strips require development by immersion for one minute in Benedict's qualitative reagent (boiling). Reducing substance is indicated by the appearance of a yellow or orange color in the filter paper strip.[1]

Fifty thousand testing units were made available to the public through drug stores in Minneapolis and St. Paul. Most of these consisted of a single Dreypak, Tes-Tape, or Clinistix unit. About 7,000 were double units consisting of a Dreypak and Tes-Tape unit stapled together. Appropriate directions were given for the four types of unit, suggesting that the urine sample to be tested be passed after a meal high in carbohydrate. The

From the Diabetes Clinic, Department of Medicine, University of Minnesota Hospitals, Minneapolis, Minnesota.

*Secretary, Lay Society, Twin Cities Diabetes Association, Minneapolis-St. Paul, Minnesota.

tests were re-inserted in identical envelopes and were distributed to the druggists in random fashion. The public was invited to make use of the tests through displays furnished for the nation-wide detection drive by the American Diabetes Association; no mention was made of the comparison being made.

An approximate estimate of tests actually distributed was made by collecting and sorting tests left with the druggists after the detection week.

Interpretation of the Tests: Test strips were returned by mail to the detection center, where they were read two days to two weeks after mailing. Reading of the enzyme strips was done by physician and lay members of the diabetes association. The main question for which an answer was sought was this: Is the sensitivity of the enzyme tests so great that an excessive number of false positive results will be obtained in comparison with a standard detection method (the Dreypak)? For this reason it was felt necessary to rate even doubtful or borderline tests as positive. Any blue or green coloring in the test strip was rated as a positive test even if it amounted to only a slight streaking. Further, all tests which were rated as positive at home were counted as positives, even though the color had faded afterward. All doubtful tests were checked by one of the authors.

The results were therefore deliberately weighted to give a maximum number of false positive tests. The effects of self-interpretation, of late development and of fading could not be estimated individually but may be assumed to have had a bearing on the results as well.

All Dreypak tests were processed in Benedict's qualitative reagent according to the technic recommended by the American Diabetes Association—by one technologist who had no knowledge of the results of the enzyme tests on any of the subjects. The Dreypaks were interpreted according to the recommendations of Dr. Drey and the Association.

Follow-Up Glucose Tolerance Tests: Everyone submitting a urine test found to be positive was offered a modified glucose tolerance test without charge. This consisted of a venous blood sugar determination two hours after ingestion of 100 gm. of glucose in the fasting state; 50 gm. of glucose was the dose used for children under the age of twelve. The blood samples were collected in fluoride tubes which were refrigerated and analyzed within three weeks by Benedict's method.[4] Normal values in the fasting state for this method lie between 60 and 90 mg. per 100 ml. The blood sugar test was offered only to persons who stated they had no previous knowledge of diabetes.

RESULTS

The results will be presented in the form of five tables.

Acceptance by the Public, and Self-Interpretation: Table 1 gives some idea of public response in relation to the type of test used.

TABLE 1

Percentage returned of four urine test units offered to the public

	Approximate number of tests distributed (Taken from druggists)	Returned to Detection Center	
		Number	Per cent
Dreypak	16,000	6,088	38
Tes-Tape	16,000	3,000	19
Clinistix	11,000	1,790	16
Double test (Dreypak and Tes-Tape)	7,000	1,687	25
All tests	50,000	12,565	25

Many people undoubtedly knew that a blue or green color on the enzyme strip indicated the presence of sugar, though no mention of this fact was made in the testing unit. Presumably some negative tests were not mailed back. The Dreypak, which had to be mailed in for processing, showed a much higher percentage return.

The likelihood that a certain amount of self-reading of the enzyme tests occurred is also shown in table 2 by the percentage of positive results yielded by the various tests:

TABLE 2

Percentage positive of urine tests returned, according to the type of test used*

	Number of Positive Tests	Per cent Positive of Tests Returned	Approximate Per cent Positive of Tests Distributed
Dreypak	233	3.8	1.5
Tes-Tape	204	6.8	1.3
Clinistix	75	4.2	0.7
Double test (Dreypak and Tes-Tape)	142	8.4	2.0
All tests	654	5.2	1.3

*Subjects already known to have diabetes excluded.

The percentage of positive tests among Tes-Tapes returned was nearly twice that among Dreypaks returned. It is important to note, however, that in terms of percentage of tests *distributed* to the public, the number of positives picked up by Tes-Tape is, if anything, a little

lower than the number detected by Dreypak.

The same effect is seen when the over-all results of the 1956 drive are compared with previous results in Minneapolis (table 3). The 1950 campaign was carried on with liquid samples (without preservative) and Benedict's qualitative test; the other drives used the Dreypak. Though the total number of tests returned in 1956 was low in comparison to most of the previous years, the percentage of positive tests was higher and the total number of positive tests returned exceeded any previous year reported.

TABLE 3

Diabetes Detection Drives in Minneapolis

Year	Total Tests Returned	Positive Tests (Including Known Diabetics) Number	Per cent
1950	24,354	534	2
1951	14,015	258	2
1952	32,063	657	2
1954	9,398	249	3
Twin Cities, 1956	12,565	728	5.8

It seems, therefore, that despite evidence of self-reading of the enzyme tests the number of positive tests reported to the center was not appreciably affected by this. The percentage positive of tests distributed was much the same for Dreypak and Tes-Tape (1.5 per cent and 1.3 per cent, respectively), making it unlikely that any appreciable number of persons, on finding a positive Tes-Tape at home, failed to mail it in. The percentage return of Clinistix tests distributed was lower (0.7 per cent); this may reflect in part the less convenient format of this test, or possibly its relative insensitivity. To be sure, even an unreported positive test will have served a major part of its purpose by calling to the subject's attention the possibility that he or she might have diabetes.

Reliability of the Enzyme Tests in Comparison with the Dreypak: The modified glucose tolerance test described above was used for evaluating the significance of the positive urine tests in persons not previously known to be diabetic. About 60 per cent of those showing glycosuria availed themselves of the test. The results were arbitrarily divided into three groups as follows: normal, 90 mg. per 100 ml. or below; borderline, 91 to 120 mg. per 100 ml.; diabetic, 121 mg. per cent or higher.

Among the double tests in which both Dreypak and Tes-Tape were used on the same specimen, one or both tests were positive for sugar in 142 specimens, but of these only twenty-eight were positive with both tests. Fifty-four tests were positive by Dreypak only and sixty by Tes-Tape only.

Eighty-three persons with one or both urine tests positive reported for blood sugar tests; the urine test results are analyzed according to these results in table 4.

In this relatively small sample, the Tes-Tape appeared to pick up a higher percentage of false positive (normal) results. More significant, probably, is the fact that both the Dreypak and the Tes-Tape missed some diabetic subjects, and in about the same proportion.

However, these differences even out when the entire group of 360 blood sugar results is analyzed in relation to the test used:

With the larger amount of data it is clear there is no difference between the sensitivity of the Dreypak, the Tes-Tape and the Clinistix.

Fading and Late-Developing Enzyme Tests: Since the interval between mailing of the enzyme strip and its reading in the detection center varied anywhere from two days to two weeks, a rough check was made on the significance of tests whose colors faded before they were read and on tests in which the color apparently had developed after being read as negative at home. It was clear in a small number of tests that a test which had faded on the way in still could mean diabetes; also that a test which was negative at home but had developed subsequently usually was normal but also could be indicative of diabetes. However, more complete observations on this point are desirable.

COMMENT

The final validity of the diagnosis depends upon repeated observations of the patient over a period of years, and there is thus some limit to the significance of a short-term study such as the present one. Within this limit, the results indicate that the enzyme tests are neither more nor less sensitive than the Dreypak for large-scale use in diabetes detection. It appears to be unlikely that the enzyme tests will produce an excessive number of false positives, or that any significant number of persons finding a positive test at home will fail to report it to the detection center. The convenience of the enzyme strips is obvious. The format of the Tes-Tape detection unit in particular was well suited to large-scale handling.

The considerable saving in effort and time that the enzyme tests allow could well be applied to the more extensive use of follow-up blood sugar tests such as were used in the present campaign.

TABLE 4

Analysis of double urine test positives according to blood sugar results in eighty-three persons*

	Dreypak Positive (Alone or with Tes-Tape)		Tes-Tape Positive (Alone or with Dreypak)		Both Dreypak and Tes-Tape Positive		All Positives	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	21	42	30	59	7	39	44	53
Borderline	15	30	8	16	1	6	22	27
Diabetic	14	28	13	25	10	55	17	20
Total	50	100	51	100	18	100	83	100

*Subjects already known to have diabetes excluded.

TABLE 5

Analysis of single and double urine test positives according to blood sugar results in 360 persons*

	All Dreypaks (Positive Alone or with Tes-Tape)		All Tes-Tapes (Positive Alone or with Dreypak)		Clinistix		All Positives (Counting Double Tests Once Only)	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Normal	78	47	68	41	24	52	163	45
Borderline	46	27	46	28	11	24	102	28
Diabetic	44	26	50	30	11	24	95	26
Total	168	100	164	100	46	100	360	100

*Subjects already known to have diabetes excluded.

SUMMARY

A comparison was made between a copper-reduction test (Dreypak) and two self-developing enzyme tests for glucose (Tes-Tape and Clinistix) in diabetes detection in Minneapolis and St. Paul. The tests were offered to the public in random fashion through drug stores. In proportion to the number of tests distributed, many more Dreypaks than self-developing tests were returned to the detection center. However, the number of *positive* tests returned in proportion to the number distributed was about the same for Dreypak and Tes-Tape and was somewhat lower for Clinistix. These results suggest (but do not prove) that some self-interpretation of the enzyme tests occurred, though it did not markedly affect the number of tests reported to the detection center.

In the most important part of the study, a modified glucose tolerance test was offered to persons showing a positive urine test; previously known diabetics were excluded. According to the results of the blood sugar tests the subjects were classified as normal, borderline, or diabetic. Of 168 subjects showing positive urine tests with Dreypak, 47 per cent, 27 per cent and 26 per cent were classified as normal, borderline and diabetic respectively. Corresponding results for 164 subjects positive by Tes-Tape were 41 per cent, 28 per cent and 30 per cent, and for forty-six subjects positive with Clinistix 52 per cent, 24 per cent and 24 per cent. These results are essentially identical and indicate that the three tests have about the same sensitivity and reliability when used in diabetes detection.

SUMMARIO IN INTERLINGUA

Le Uso De Simple Tests Enzymatic Pro Glucosa In Le Detection De Diabete: Evalutation Per Medio De Un Modificate Test Del Tolerantia Pro Glucosa

In Minneapolis e St. Paul, un comparation esseva effectuate inter un test a reduction de cupro (Dreypak) e duo auto-disveloppante tests enzymatic (Tes-Tape e Clinistix) pro glucosa in le detection de diabete. Le tests esseva offerite al publico al hasardo via le drogerias. In relation al numero de tests distribuite, le proportion del Dreypaks retornate al centro de detection esseva multo plus grande que le proportion del Tes-Tapes e Clinistixes. Tamen, le numero del tests a reaction positive que esseva retornate in proportion al numero distribuite esseva circa le mesme in le casos de Dreypak e de Tes-Tape e un pauco plus basse in le caso de Clinistix. Iste factos indica possibilemente sed non necessarimente que il occurreva un certe auto-interpretation del tests enzymatic per le individuos, sed isto non afficeva marcatemente le numero de tests reportate al centro de detection.

In le plus importante parte del studio, un modificate test de tolerantia pro glucosa esseva offerite a personas con positive tests del urina. Individuos previemente cognoscite como diabeticos non esseva includite. Super le base del resultatos del tests de sucro sanguinee, le subjectos esseva classificate como normal, casos limite, o diabetic. Ex 168 subjectos con positive tests urinari per Dreypak, le tres classes comprendeva 47, 27, e 26 pro cento, respectivemente. Le cifras correspondente pro 164 subjectos positive per Tes-Tape esseva 41, 28, e 30 pro cento. Pro quaranta-sex subjectos positive per Clinistix, illos esseva 52, 24, e 24 pro cento. Iste resultatos es essentialmente identic. Illos indica que le tres tests es plus o minus equal in sensibilitate e fidelitate quando usate in le detection de diabete.

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Theories of Hunger Sensation

Numerous ideas have been advanced to explain the induction of hunger sensations. One of the early theories, proposed by E. Bulatao and A. J. Carlson (*Am. J. Physiol. 59*:107, 1924) was based on the fact that hunger pangs coincided with the waves of contraction of the empty stomach. They also postulated that hypoglycemia was responsible for this effect. W. W. Scott, C. C. Scott and A. B. Luckhardt (*Am. J. Physiol. 123*:243, 1938) determined blood sugar on human subjects preceding, during, and after normal hunger periods, and found no variations which indicated that the blood sugar level bore a causal relation to the normal hunger periods of man.

J. Mayer (*New Eng. J. Med. 249*:13, 1953) reasoned that it was unlikely that the abundant body stores of protein or fat would decrease much between meals to stimulate the hypothalamic centers. On the other hand, the carbohydrate stores of the body, being small, could easily be depleted. He proposed that hypothalamic "glucoreceptors," sensitive to variations in available blood glucose, were responsible for initiating hunger sensations. Available blood glucose was determined by finding the arteriovenous difference. Since measurement of the arteriovenous blood sugar difference was not possible in the hypothalamic region, the determinations were carried out on peripheral blood with the assumption that the findings were representative of the body generally. Correlation of these findings with hunger periods in subjects on different dietary regimens showed that, on a calorically adequate diet, the arteriovenous (a-v) difference remained high during the day, decreasing only at meal time along with the appearance of hunger feelings. In contrast, hunger appeared earlier on submaintenance diets and the glucose differences became rapidly smaller. However, a recent review on the role of blood sugar and appetite (*Nutrition Reviews 14*:332, 1956) urges caution in the general interpretation of peripherally determined a-v sugar differences.

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