Tests for Glucosuria

An Analysis of Factors That Cause Misleading Results

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SUMMARY

The incidence of potentially erroneous tests for urinary glucose was studied. Each urine specimen collected was tested with Clinitest, Clinistix, Diastix, and Tes-Tape before and after addition of glucose to produce a urine glucose concentration of 1/2 per cent. There was a 23 per cent incidence of falsely high (>1/2 per cent) and a 33 per cent incidence of falsely low (< 1/2 per cent) results in the 513 specimens examined. Underreading, frequently caused by urinary metabolites of common medications, was seen only with Clinistix and Diastix. Overreading had a distinct relationship to dilute urine and was most frequent with Clinitest (65 per cent). The patient's habits (medications taken and water ingestion) should be considered carefully when a urine testing method is selected. Periodic plasma glucose measurements are recommended to confirm impressions obtained from urine tests for glucose. DIABETES 22:115-21, February, 1973.

Accurate tests for urinary glucose are essential to management of patients with diabetes mellitus. The results of these tests are used to adjust the quantity, type, and frequency of insulin or oral hypoglycemic therapy.

The false positive Benedict test for glucose, produced by medications (salicylates, ascorbic acid) or metabolites (fructose, homogentisic acid) is well known. We recently reported that false negative glucose oxidase tests in the presence of glucosuria may occur in patients ingesting aspirin and L-dopa (L-3,4 dihydroxyphenylalanine) or in patients with alcaptonuria.[1] This false negative test is produced by potent reducing metabolites in the urine (gentisic, 3,4 dihydroxyphenylacetic, homogentisic or 5-hydroxyindole acetic acids) which keep the indicator dye in the strip of test paper in a reduced form.

The present study was designed to determine the frequency with which misleading tests for glucose occur among patients in a general hospital and clinic population who receive a variety of medications.

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MATERIALS AND METHODS

In preliminary studies, twenty-five urine samples free of glucose as tested by Tes-Tape (Lilly), Clinistix (Ames), Diastix (Ames), and Clinitest (Ames) were obtained from normal volunteers. The specimens were then fortified with glucose to a concentration of 0.05 per cent and retested. This small amount of glucosuria produced a positive test with Tes-Tape in 100 per cent of the specimens, with Diastix in 40 per cent, and with Clinitest in 20 per cent. The glucosuria was not evident with Clinistix. Therefore, urine samples that did not react with Tes-Tape on the basal test were judged to be free of significant glucose for the purposes of the present study.

Urine specimens were obtained from 513 diabetic and nondiabetic patients at Duke University Medical Center. Of the total collected, 313 were from hospitalized patients, 95 from patients seen in the Duke Diabetes Clinic, and 105 from patients seen in the general medical and surgical clinics. On the morning of collection each urine specimen was immediately taken to the laboratory and tested, by the investigators, for glucose using Tes-Tape, Clinitest, Clinistix, and Diastix (basal test). Those urine specimens that reacted with Tes-Tape or Clinitest were judged to contain glucose, or a nonglucose reducing substance, and were deemed "positive." Each specimen was then fortified with a 5 per cent glucose solution (so that the concentration of urinary glucose became ½ per cent or greater) and the tests were repeated (fortified test). This concentration of glucose was chosen because it produces an easily distinguishable color with all four tests.

All urine specimens that tested less than the predicted ½ per cent, after addition of glucose, were acidified to a pH of 3 (with 5 N hydrochloric acid) and frozen for later, more detailed analyses. Some samples that tested higher than ½ per cent were also frozen (without ad-

dition of acid) for further analyses.

Semiquantitative determination of reducing substance with Clinitest tablets depends on the color change produced when cupric sulfate is reduced to cuprous oxide by any appropriate reducing agent. In contrast, enzyme tests, such as Clinistix, Diastix, and Tes-Tape contain glucose oxidase, peroxidase, and chromogen which respond specifically to glucose (figure 1). In Clinistix and Diastix, the three testing materials are located in a small test area on one end of a cellulose strip; in Tes-Tape they are impregnated throughout a paper strip.

identified by its characteristic fluorescence with ultraviolet light and its ability to reduce silver after spraying with an ammoniac silver nitrate solution. The amount of gentisic acid present was estimated by comparing the size and intensity of the silver reduction spots from the urine extracts with the silver reduction spots of the known standards.

Urinary ascorbic acid was measured by a method that depended on the reduction of the indicator, 2,6 dichlorophenolindophenol, as read on a Bausch & Lomb spectrometer.[2] This indicator dye was not decolorized by other

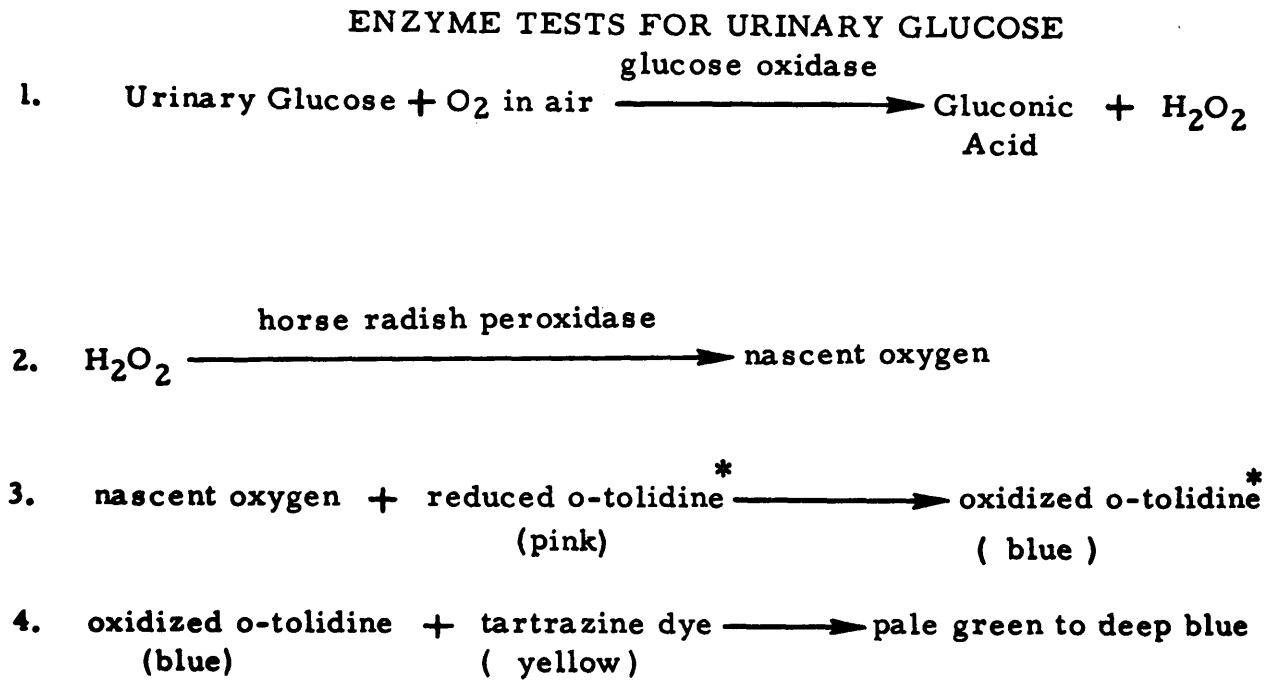

FIG. 1. Chemical reactions occurring in glucose oxidase paper tests. Clinistix utilizes the first three reactions. In Tes-Tape the dye, tartrazine, is added to give a greater range of colors (reaction 4).

Thin layer chromatography was used to determine the urinary metabolites responsible for falsely low tests for glucose.[1] Although many of the urine specimens showed a number of unknown spots, the most consistent compound noted was gentisic acid, a potent reducing metabolite of salicylates. Semiquantitative estimation of the gentisic acid content was made by acidifying 6 ml. of urine to pH 1, extracting it with 2 ml. of diethyl ether, and applying 25 μL. to silica gel G plates. Varying concentrations of a gentisic acid standard were extracted into ether from aqueous standard solutions and chromatographed simultaneously. The plates were developed with butanol saturated with water. Gentisic acid was

reducing substances, such as gentisic acid or 5-hydroxyindole acetic acid.

Urine osmolality was determined by the freezing point depression methods, using an Advanced Instruments osmometer. Urinary urea and creatinine were measured by standard methods.[3,4]

RESULTS

Of the 513 total urine specimens tested, 389 tested negative with Tes-Tape on the basal test; 110 tested positive on Tes-Tape and/or Clinitest, and fourteen produced colors different from those on the Clinitest Color Chart and were excluded from the study group as uninterpretable.

Falsely low tests for glucose. Table 1 illustrates the results of testing the 389 fortified urine specimens that were negative on the basal test. Falsely low readings were seen in eighty-five specimens: 19 per cent underread with Clinistix; 25 per cent with Diastix; and 56 per cent with both Clinistix and Diastix. None underread with Clinitest or Tes-Tape.

TABLE 1

Validity of urine tests for glucose in 389 fortified specimens negative on basal tests with Tes-Tape

	No Error	Underread	Overread
In-patients	76 (30%)	59 (23%)	118 (47%)
Diabetes clinic	25 (44%)	11 (20%)	20 (36%)
General clinics	31 (39%)	15 (19%)	34 (42%)
	132 (34%)	85 (22%)	172 (44%)

Specimens that reacted with Clinitest and/or Tes-Tape on the basal test were judged to contain glucose or a nonglucose reducing substance (table 2). Of the 110 samples in this category, thirty-five underread on the fortified tests; of these thirty-five specimens, eighteen were positive with Clinitest only and underread on the Clinistix and Diastix after glucose fortification. This suggested that they originally contained a potent nonglucose reducing substance. Of the thirty-five urine specimens which underread on the fortified tests, 17 per cent underread on Clinistix, 23 per cent on Diastix, and 60 per cent on both Clinistix and Diastix. None of these urines underread on Clinitest or Tes-Tape.

TABLE 2

Validity of urine tests for glucose in 110 fortified specimens that were positive on basal tests with Clinitest and/or Tes-Tape

	No error	Underread
In-patients	26 (54%)	22 (46%)
Diabetes clinic	31 (80%)	8 (20%)
General clinic	18 (78%)	5 (22%)
	75 (68%)	35 (32%)

An agent responsible for the falsely low test for glucose could be identified in 51 per cent of the 120 specimens that underreacted on the fortified tests (figure 2). By adding variable amounts of ascorbic and gentisic acids to a normal urine, it was established that the minimal concentration of these chemicals which consistently produced a falsely low reaction with the Clinistix test were ascorbic acid, 0.09 mg./ml., and gentisic acid, 0.05 mg./ml. Therefore, when the urines tested contained these concentrations of either acid, the falsely low reaction could be attributed to these substances. Of the 120 urine samples which underreacted for glucose, 16 per cent contained at least 0.09 mg./ml. ascorbic acid, 29 per cent contained at least 0.05 mg./ml. gentisic acid, and 6 per cent contained more than 0.09 mg./ml. ascorbic acid and 0.05 mg./ml. gentisic acid.

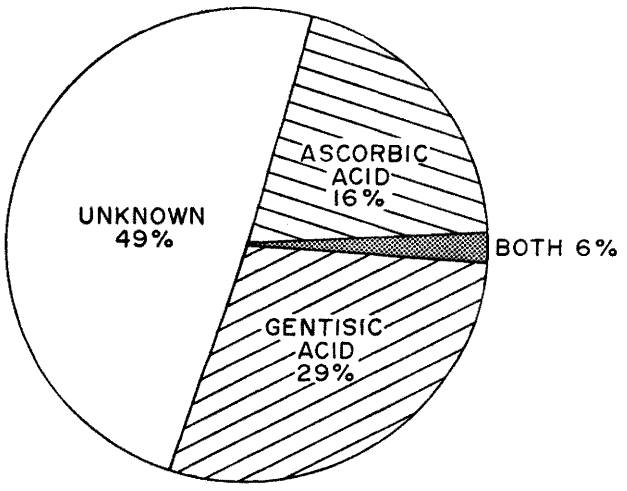

FIG. 2. Distribution of factors that cause falsely low tests for urinary glucose in tests of specimens fortified with glucose.

The records of patients whose urine underreacted were reviewed for all current medications. Only 29 per cent of the patients whose urine contained 0.09 mg./ml. of ascorbic acid had vitamin C or medications containing vitamin C recorded. Of the urines which contained 0.05 mg./ml. gentisic acid, 56 per cent had aspirin or medications containing aspirin recorded.

During the chromatographic identification of gentisic acid, numerous unidentified reducing compounds were seen. These compounds were probably metabolic products of the diverse medications the patients were receiving. During review of the records, all prescribed medications were tabulated. Table 3 illustrates the sixteen medications most frequently recorded on the patients' charts.

Table 4 shows test results for two patients with metastatic carcinoid tumors and the carcinoid syndrome. Initially, both patients had 5-hydroxyindole acetic acid (5-HIAA) levels greater than 0.25 mg./ml. of urine, and the Clinistix and Diastix underestimated the urinary glucose. We have demonstrated previously that 5-HIAA in amounts of 0.25 mg./ml. inhibits color development on the enzyme tests.[1] After the urinary 5-HIAA levels were reduced by streptozotocin therapy,[5] the tests again became accurate.

Falsely high tests for glucose. During the testing of

TABLE 3

Medications taken by patients whose urine specimens contained substances that caused underreading when fortified with glucose

Trade name	Generic name	Per cent of total patients receiving the medication
Aspirin	Aspirin	14
Allbee with C Berocca C Reavita	Ascorbic acid	10
Lanoxin	Digoxin	10
Prednisone	Prednisone	10
Darvon Compound	Propoxyphene HCl, aspirin, phenacetin, caffeine	9
Dulcolax	Bisacodyl	9
Ferrous Sulfate	Ferrous sulfate	8
Valium	Diazepam	8
Ampicillin	Ampicillin	7
Dalmane	Flurzaepam HCl	7
Ecotrin	Aspirin	6
Multivitamins	Multivitamins	6
Seconal	Secobarbital	6
Chloral Hydrate	Chloral hydrate	5
Lasix	Furosemide	5
Phenobarbital	Phenobarbital	5

fortified urine specimens, it was noted that many tested above the expected ½ per cent. Of a total of 389 samples, which were negative on basal tests, 172 gave a falsely high response (table 1). Clinitest tablets were most often associated with overreading, and falsely high tests occurred in 111 of the 172 specimens (65 per cent). Of these 111 specimens, eighty-nine tested at ¾ per cent and twenty-two tested at 1 per cent.

Many specimens that tested falsely high were pale in appearance. Their osmolality was usually lower (< 100 m. osmols/kg.) than the osmolality of the urine specimens that accurately reflected glucose content. To evaluate the effect of osmolality on tests for urinary glucose, a normal volunteer drank 1,900 cc. of water over a one-hour period. Serial urine specimens were collected and basal and fortified tests were carried out to determine glucose content as described above. Aliquots of each specimen were analyzed for osmolality, urea content, and creatinine content.

TABLE 4

Inhibition of urine tests for glucose in specimens obtained from patients with the carcinoid syndrome (fortified tests)

Patient	5-HIAA (mg./ml.)	Clinitest	Tes-Tape	Clinistix	Diastix
Control patient	0.005	1/2%	1/2%	Medium	1/2%
W.P. #1	1.37	trace*	1/2%	Light	1/4%
W.P. #2	0.22	1/2%	1/2%	Medium	1/2%
J.P. #1	0.37	1/2%	1/2%	Light	1/4%
J.P. #2	0.11	1/2%	1/2%	Medium	1/2%

*Took two-and-one-half minutes for Clinitest tablet to completely react.

On the basal test, all urine specimens were negative for glucose. As the induced diuresis progressed, there was a striking difference in the results of the fortified tests (table 5). Prior to the onset of diuresis, the tests all accurately reflected the actual glucose concentration (½ per cent). With the onset of the brisk diuresis (specimen 4), all four tests overread the glucose content of the urine, and this persisted until the diuresis abated and urine osmolality rose to 486 m. osmols/kg.

When glucose (½ per cent) was added to water and tested, it reacted as dilute urine and read 1 per cent on all four tests. To determine if overestimation was due to viewing the precipitate of cuprous oxide through the light yellow liquid of a dilute urine, a dilute fortified urine was reacted with a Clinitest tablet and read 1 per cent. The cuprous oxide precipitate was separated by centrifugation and the supernatant replaced with the supernatant of a concentrated urine. Despite this replacement with the deep yellow urine, the precipitate continued to read 1 per cent, indicating that overestimation was not due to the decreased pigment of a dilute urine.

If a critical factor in the overestimation of glucose in dilute urines is decreased concentration of an essential urinary constituent, replacement of such a constituent should correct the testing error. Sodium chloride was added to aliquots of each specimen to restore osmolality to 744 m. osmols/kg. (as found in specimen 1). Persistent overestimation of glucose remained on both the Clinitest and enzyme tests. Addition of enough sodium chloride to raise osmolality to 2,400 m. osmols/kg. did not correct the Clinitest overreading but did correct the testing error on the enzyme tests. In a similar manner, urea and creatinine were added to aliquots of each specimen to match the urea and creatinine levels found in specimen 1. These maneuvers, and the addition of excessive amounts of uric acid, did not correct the Clinitest or enzyme tests.

The brisk water diuresis described above would not ordinarily occur in patients with diabetes. However, diabetic patients do undergo a mild diuresis when obtaining a double-voided urine specimen. The patient is advised to empty his bladder, discard the urine, drink

TABLE 5

Effect of a water load on urine testing for glucose in a normal subject

Specimen	Urine Characteristics				Fortified Urine Tests			
	Volume (cc./min.)	Osmolality (m. osmols)	Urea (mg./ml.)	Creatinine (mg./ml.)	Clinitest (%)	Tes-Tape (%)	Clinistix	Diastix (%)
1	2.1	744	7.00	1.25	1/2	1/2	Medium	1/2
2	4.1	298	1.86	0.45	3/4	1/2	Dark	1/2
3	13.5	85	0.78	0.13	1	2	Dark	1/2
4	16.2	69	0.64	0.12	1	2	Dark	1
5	14.6	61	0.63	0.16	1	2	Dark	1
6	13.4	58	0.68	0.18	1	2	Dark	1
7	12.5	59	0.68	0.13	1	2	Dark	2
8	14.5	74	0.83	0.13	1	2	Dark	2
9	2.0	374	3.84	0.70	3/4	1/2	Dark	1
10	1.6	486	6.00	1.01	1/2	1/2	Medium	1/2

two glasses of water, void in one-half hour, and test this second-voided specimen for glucose. To determine if a mild water-induced diuresis could affect tests for urinary glucose, nine healthy volunteers gave random urine specimens. They then drank two 8-oz. glasses of water and voided a second specimen thirty minutes later. The specimens were tested for glucose as described above, and urine osmolality was measured. All the specimens, whether obtained before or after water ingestion, were free of glucose. Glucose was then added to all samples to achieve a known final glucose concentration of ½ per cent.

Table 6 illustrates the results of this experiment. After the addition of glucose, the first-voided urine specimens of eight of the nine subjects tested with Clinitest accurately reflected the true glucose content. The falsely low results with Clinistix and Diastix could not consistently be accounted for. However, subjects 3 and 4 had sufficient ascorbic acid in their urine to account for such an underreaction ($>$ 0.09 mg./ml.). When the second-voided specimens were tested and the results compared to those of the first-voided specimens, higher glucose readings were obtained in some of the second-voided urines by each of the methods (Clinitest 80

TABLE 6

Differences in "first" and "second" voided specimens on tests for urinary glucose

Subject	Specimen	Urine Characteristics		Fortified Urine Tests			
		Osmolality (m. osmols)	Ascorbic Acid (mg./ml.)	Clinitest (%)	Tes-Tape (%)	Clinistix	Diastix (%)
1	F	1,025	0.023	1/2	1/2	Light	1/2
	S	825	0.030	3/4	1/2	Light	1/4
2	F	680	0.011	1/2	1/2	Medium	1/2
	S	274	0.006	3/4	2	Dark	1
3	F	522	0.156*	1/2	1/2	Light	1/2
	S	260	0.091*	3/4	1/2	Light	1/2
4	F	1,013	0.090*	1/2	1/2	Light	1/4
	S	1,094	0.120*	3/4	1/2	Light	1/4
5	F	1,134	0.013	1/2	1/2	Light	1/4
	S	736	0.016	3/4	1/2	Medium	1/2
6	F	1,000	0.041	1/2	1/2	Light	1/4
	S	869	0.033	3/4	1/2	Light	1/4
7	F	764	0.026	1/2	1/2	Medium	1/2
	S	411	0.018	3/4	2	Dark	1
8	F	944	0.012	1/2	1/2	Medium	1/2
	S	423	0.006	3/4	2	Dark	1
9	F	315	0.006	3/4	1/2	Medium	1
	S	108	0.002	1	2	Dark	2

F = first-voided urine specimen
S = second-voided urine specimen
* = sufficient ascorbic acid to account for underreaction

per cent; Clinistix 56 per cent; Diastix 44 per cent; and Tes-Tape 44 per cent). The Clinitest determination of subject 9's first-voided urine was already in error (¾ per cent) and the error became greater in the second-voided specimen (1 per cent). She had the lowest initial urinary osmolality.

This experiment shows there can be a significant overestimation of urinary glucose content in second-voided samples. Although all four of the standard tests tend to overestimate glucose content in second-voided specimens, the Clinitest method appears most liable to overestimation.

COMMENTS

This study indicates that the incidence of potentially misleading urine tests for glucose is high. After glucose fortification of urine specimens, testing procedures resulted in correct estimation in 40 per cent, uninterpretable results in 3 per cent, underestimation in 23 per cent (with Clinistix and Diastix), and overestimation in 34 per cent of the specimens tested. Misleading tests were due to intrinsic properties of the urine: underestimation to reducing compounds and overestimation to some alteration found in dilute urine. Therefore, intrinsic alterations in urine (as well as previously recognized factors of high renal threshold for glucosuria and improperly performed testing procedures) must be considered as an important cause of misleading tests for glucosuria.

The most frequent cause of underreading was the presence of reducing compounds, such as ascorbic acid and gentisic acid, in the urine. Aspirin and ascorbic acid were not recorded on the charts of many patients who had these compounds in their urine specimens. Ascorbic acid is a nonprescription compound frequently used for prevention of respiratory infections.[7] Many fruit drinks and foods are fortified with this compound, as it acts as a preservative and antioxidant. Multivitamin tablets contain generous amounts of the compound. It is present in many nonvitamin medications; for example, intravenous tetracycline preparations (such as Achromycin and Tetracyn) are buffered with up to 2,000 mg. of ascorbic acid/500-mg. dose of antibiotic; and it is found in iron preparations, such as Feosol.

Aspirin, the precursor of gentisic acid, is found in almost all proprietary pain medications. Casual doses of aspirin will probably not result in misleading tests, for only 1 to 8 per cent of the aspirin is converted to its potent reducing metabolite, gentisic acid. We have previously noted that 50 per cent of patients taking 2.4 gm. of aspirin per day have enough gentisic acid in their urine to affect the measurement of glucosuria.[1] Patients taking regular doses of aspirin for chronic conditions such as arthritis, headache, or rheumatic fever do excrete adequate gentisic acid to affect their urine tests.

The most significant factor in overestimation of glucosuria seems to be dilute urine. Physicians may inadvertently encourage such overestimation by instructing their patients in the second-voided urine technic. It is important that this potential error be kept in mind if the Clinitest method is used to estimate glucosuria in pooled six or twenty-four hour urine collections.[8] If the pooled specimen were dilute, this method could result in 100 per cent overestimation of glucosuria.

One might ask, in the light of these data, what is the best urine testing method. Clinitest does not give falsely low tests in the presence of small amounts of reducing substances. It is fairly easy to interpret over its entire range of colors (although we did find 3 per cent of the specimens were uninterpretable due to color distortion). It is the most likely to overestimate glucose in dilute urines and it gives falsely positive tests in the presence of large amount of nonglucose reducing compounds. Clinistix and Diastix have the highest incidence of falsely negative results in the presence of even smaller amounts of reducing substances. The semiquantitative interpretation of Clinistix is difficult but is quite clear with Diastix.

Tes-Tape is the most sensitive test for glucose, consistently detecting as little as 1/10 per cent glucose[9] or 1/20 per cent glucose, as shown in the present study. It is the least affected by reducing metabolites. We have previously suggested that Tes-Tape is able to avoid falsely negative tests since it acts as a minichromatography system, separating glucose from reducing metabolites. Its sensitivity for glucose and its resistance to underreading make Tes-Tape a very satisfactory preparation both for screening for diabetes and for following the adult-onset diabetic patient whose disease is fairly well regulated. It is less useful in care of patients with brittle or juvenile diabetes because the discrimination of shades of green (with glucose concentrations greater than ½ per cent) is sometimes difficult to make.

The present study indicates that the potential for misleading tests for glucosuria exists in a significant number of patients in a general medical population. Physicians and patients should be alert to the various factors which contribute to such misleading tests. Errors in treatment of diabetes may occur if only results of urine tests are relied upon.

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