

Inhibition of Glucose Oxidase Paper Tests by Reducing Metabolites

Jerome M. Feldman, M.D., William N. Kelley, M.D., and Harold E. Lebovitz, M.D., Durham

SUMMARY

A negative urinary glucose oxidase test in the presence of glucosuria may occur in patients with alcaptonuria or the carcinoid syndrome. It may also be noted after ingestion of aspirin or L-dopa. The responsible agents are potent reducing metabolites such as gentisic acid, homogentisic acid, or 5-hydroxyindole acetic acid. These agents produce the misleading reaction by keeping the indicator dye o-tolidine in its reduced form. In the presence of such reducing agents the tests for glucosuria should be carried out either with Tes-Tape or by modifying the manufacturer's instructions and only partially immersing the Clinistix strip. *DIABETES* 19:337-43, May, 1970.

The false positive Benedict test for urinary glucose produced by homogentisic acid, medications such as salicylates or by the presence of other reducing sugars in the urine is well known. The nonspecificity of these reactions is usually shown by retesting the urine with one of the more specific glucose oxidase enzyme paper tests. A negative reaction with that test is usually accepted as "prima facie" evidence that glucosuria is not present.

We have recently studied a patient with concurrent diabetes mellitus and alcaptonuria. Though this patient had significant hyperglycemia (blood glucose 300 to 400 mg./100 ml.) and polyuria, the urinary glucose oxidase paper strip test (Clinistix) was consistently negative. It was demonstrated that this was a misleading laboratory artifact, and was due to the presence of a potent reducing agent, homogentisic acid, in the urine.¹ The investigations reported here indicate that false negative glucose oxidase paper tests for urinary glucose are not restricted to patients with this rare genetic disease, but occur in patients taking a variety of diverse and commonly used drugs such as aspirin or L-dopa (L-3,4 dihydroxyphenylalanine). Ingestion of these drugs results in the urinary excretion of potent reducing agents, and it is these reducing agents that are responsible for the false negative tests.

From the Department of Medicine, Division of Endocrinology, Duke University Medical Center, Durham, North Carolina 27706.

MATERIAL AND METHODS

Patients

The patient with alcaptonuria was a forty-four-year-old Caucasian man whose main complaints were related to arthritis and poorly controlled diabetes mellitus. His fasting blood glucose was 328 mg./100 ml. and his twenty-four-hour urinary excretion of homogentisic acid was 1,921 mg./day (Bioscience Laboratory).

The sixteen patients receiving aspirin (five men and eleven women) ranged in age from seventeen to sixty-five years. Although the majority of patients were receiving aspirin for rheumatoid arthritis, some were being treated for other inflammatory or febrile conditions. Although many were taking additional medications, it was ascertained that none of the other medications influenced the urinary glucose oxidase test in the absence of aspirin ingestion.

The patients receiving L-dopa were being treated for Parkinson's syndrome and they ranged in age from forty-five to seventy years old. Forty-two individual urine specimens were examined from the twelve men and women studied. L-dopa dosage was increased at two-day intervals and, therefore, many of the patients were studied at different dose levels. The specimens were obtained on the second day of a given dose level.

Urine tests

No attempt was made to control the patients' fluid intake, and random urine specimens were obtained at various times throughout the day. Urinary pH was determined with nitrazine paper and ranged from a pH of 5 to a pH of 7. The urine was tested with Clinitest Tablets (Ames), Clinistix (Ames), and Tes-Tape (Lilly). The semiquantitative determination of reducing substance with Clinitest tablets depends on the color change produced when cupric sulfate is reduced to cuprous oxide. This reduction of copper is produced by any appropriate reducing agent. In contrast, the Clinistix and Tes-Tape systems contain glucose oxidase, peroxidase and chromogen. Both of these preparations respond specifically to the presence of glucose. In Clinistix, the three testing materials are located in a small test area on one end of a cellulose strip, while in Tes-Tape they are impregnated throughout a paper tape.

For studying the inhibition of the glucose oxidase paper tests, 0.1 ml. of 10 per cent glucose was added to 0.9 ml. of urine and the Clinitest, Clinistix and Tes-Tape reactions of this 1 per cent glucosuria specimen were carried out in accordance with the instructions of the manufacturer. This concentration of glucose in control urine resulted in a dark Clinistix reaction. Treatment urines that gave a negative or light reaction despite the presence of 1 per cent glucose were progressively diluted with control urine containing 1 per cent glucose until a medium or dark reaction was noted. This furnished a semiquantitative estimate of the quantity of the interfering material present in the urine.

In additional studies, various known substances were added to control urine that had been adjusted to a 1 per cent glucose concentration. Once again the Clinitest, Clinistix and Tes-Tape reactions were carried out. Decreasing concentrations of interfering substances were added until the end point of a medium to dark Clinistix reaction was obtained.

Chemicals

L-dopa, L-tryptophan, 5-hydroxytryptophan (5HT), 5-hydroxyindole acetic acid-cyclohexyl ammonium salt (5-HIAA), serotonin-creatinine sulfate salt, homovanillic and vanilly-mandelic acid (VMA), tyrosine and phenylalanine were purchased from Ca Biochemical Company. Homogentisic acid, caffeine, xanthine, glucuronic acid, β -phenylpyruvic acid, and 5-hydroxyindole acetic acid were purchased from Sigma Chemical Company. Genticic acid was purchased from Mann Biochemical.

RESULTS

Alcaptonuria

In alcaptonuria there is a deficiency of homogentisic acid oxidase that results in impairment of oxidation of the intermediary metabolite homogentisic acid.² This substance, a normal metabolite of phenylalanine and tyrosine, is therefore excreted in the urine (figure 1—1a and b).

To evaluate the possibility that an inhibitor of the glucose oxidase reaction was present in the urine of the patient with alcaptonuria, his urine was fortified with an additional 1 per cent concentration of glucose. Despite this addition of glucose, the urine continued to give a negative Clinistix reaction. Mixing experiments demonstrated that 0.25 ml. of alcaptonuric urine could inhibit Clinistix color formation when added to 2 ml. of normal urine when the mixture was adjusted to a 1 per cent glucose concentration, confirming the presence of an inhibitory substance. Further studies indicated that as little as 0.05 mg./ml. of homogentisic

acid could inhibit the positive Clinistix reaction of a 1 per cent glucose in urine. Thus, homogentisic acid is as effective an inhibitor as the known reducing agent ascorbic acid,³ which in the present studies had a minimal inhibitory concentration of 0.1 mg./ml.

Homogentisic acid is also present in the blood of patients with alcaptonuria while this metabolite is not measurable in the plasma of normal individuals. To see if the plasma levels found in alcaptonuria will interfere with the enzymatic determination of blood glucose, a glucose tolerance test was carried out on the patient. Blood samples were analyzed for glucose by both the autotechnicon ferricyanide method,⁴ and the enzymatic glucose oxidase method.⁵ The glucose values obtained by the two methods were in essential agreement. This is probably because the blood homogentisic acid levels are not as strikingly elevated, as are the urinary levels of this metabolite.

Salicylates

The effect of aspirin ingestion on the glucose oxidase urine reaction was investigated. Aspirin was selected because the ingestion of salicylates results in the urinary excretion of a compound structurally related to homogentisic acid, gentisic acid (figure 1—2a and b).

After adjustment to 1 per cent glucose concentration, five of the ten urine specimens from patients ingesting 2.4 gm. to 2.7 gm. of acetylsalicylic acid per day inhibited the Clinistix reaction, while five out of seven specimens from patients receiving 3.6 to 5.4 gm. of aspirin per day inhibited this reaction.

Gentisic acid 0.1 mg./ml. was added to normal urine that had previously been adjusted to a 1 per cent glucose concentration. This urine then gave a negative Clinistix test for glucose, demonstrating that gentisic acid is as potent an inhibitor of the glucose oxidase reaction as is homogentisic acid. In contrast, the major excretory product of aspirin, sodium salicylate, had no effect on the glucose oxidase reaction, even when added to normal urine in concentrations of 10 mg./ml. A saturated solution of aspirin itself was also without effect on this reaction.

The pH of the urine specimens obtained from patients ingesting salicylates was elevated to 8.5 with sodium hydroxide, and the open containers were allowed to stand overnight at room temperature. Many of the urine specimens gradually darkened downward from the surface until the entire sample was dark brown. A similar type of darkening occurred when normal urine alkalized and fortified with 2 mg./ml. of gentisic acid was allowed to stand for several hours. There was a good correlation between the degree of

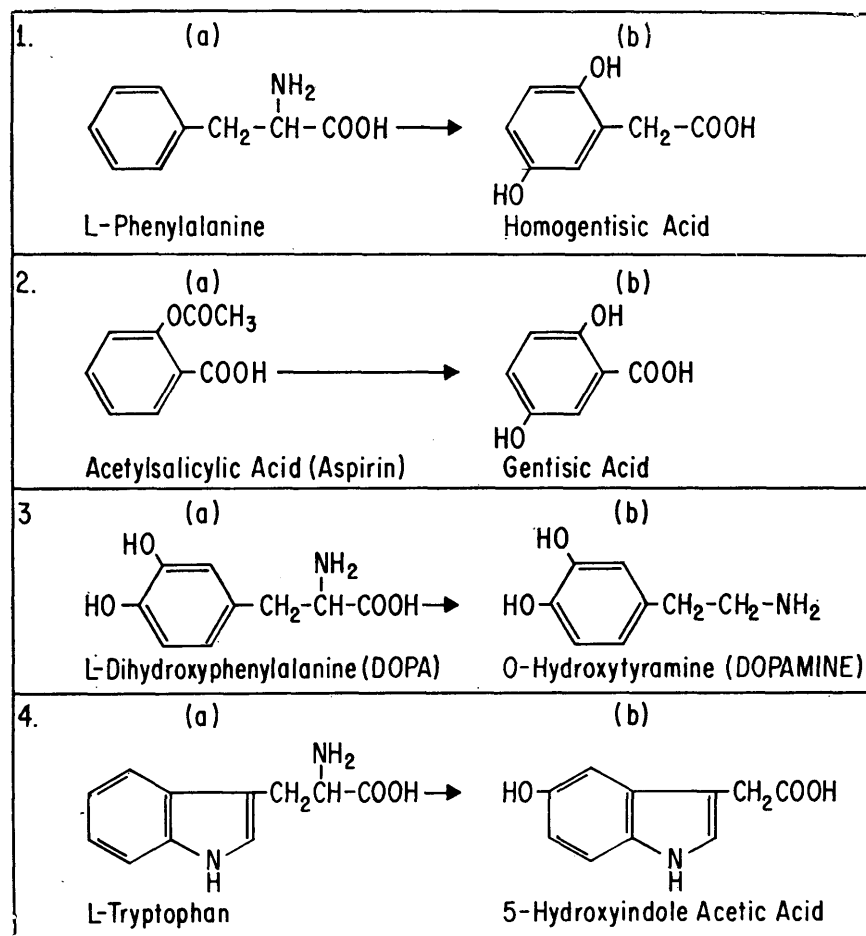


FIG. 1. The origin of some reducing materials found in the urine that interfere with the glucose oxidase reaction.

darkening of a urine and its ability to produce a false negative Clinistix reaction.

The presence of significant quantities of gentisic acid in the urine of representative patients producing a false negative Clinistix test was confirmed by thin layer chromatography. After acidification, the urine specimens were extracted with diethyl ether and applied to silica gel G plates along with reference standards of gentisic acid, homogentisic acid, sodium salicylate and acetylsalicylic acid. Chromatography was carried out in two different solvent systems (butanol saturated with water and 80 per cent saturated ammonium sulfate with 2 per cent v/v isopropanol). The plates were sprayed with diazotized sulfanilic acid or ammoniacal silver nitrate. Staining with the latter material would indicate a reducing agent. A single reducing spot was present in each case examined, and the migration of this spot was identical with the gentisic acid standard (R_f , butanol-water 0.86, ammonium sulfate-isopropanol 0.74).

Although the inhibition of the Clinistix reaction increased with increasing salicylate ingestion, there were

some exceptions noted. One patient was receiving 2.7 gm. of aspirin per day when a 1:8 dilution of her urine effectively inhibited the Clinistix reaction. In contrast the undiluted urine of another patient receiving 4.8 gm. of salicylate/day did not inhibit the reaction. Eight of the ten inhibitory urine specimens continued to inhibit the reaction even after a dilution of 1:2 or greater, with normal urine. Although most of the urines producing a false negative Clinistix reaction also produced a false positive Clinitest reaction, on occasion the former occurred in the absence of the latter. This may be due to varying quantities of salicylate-glucuronic acid conjugates, for these conjugates may be capable of reducing Clinitest tablets, but not potent enough to affect the Clinistix reaction.

L-dopa

In an earlier study it was noted that the direct addition of large quantities of epinephrine (1 mg./5 ml.) to urine specimens would produce a false negative glucose oxidase reaction.⁶ The recent introduction of L-dopa in the treatment of Parkinson's disease furnished

us the opportunity to examine the urine of patients receiving gram quantities of catecholamine precursors, and it was conceivable this group of patients might have reducing substances in their urine.

In the group of patients receiving 0.75 gm. to 3.0 gm. of L-dopa, six out of twenty-five specimens reduced the Clinitest copper reagent in the absence of glucosuria. After adjustment of the urines to a 1 per cent glucose concentration, the same urine specimens produced a false negative Clinistix reaction. In the higher dose range of 3.5 to 5.0 gm./day, thirteen out of seventeen specimens reduced the Clinitest copper reagent, and after adjustment of the glucose concentration to 1 per cent the same thirteen specimens also inhibited the Clinistix glucose oxidase reaction.

The principal known urinary metabolite of L-dopa is homovanillic acid (HVA) and 23 per cent of the L-dopa ingested is recovered in the form of this compound.^{7,8} Much smaller quantities of L-dopa are recovered from the urine as dopamine (o-hydroxytyramine) and L-dopa itself (figure 1—3a and b). However, the metabolic fate of at least 70 per cent of the ingested L-dopa has not yet been determined. HVA, dopamine, and other urinary products of monoamine excretion were evaluated for their ability to produce a false negative Clinistix reaction. The results obtained when these substances were added to control urine containing 1 per cent glucose are tabulated in table 1. Dopamine, the most potent of these agents in inhibiting the Clinistix reaction, had only about one-sixteenth the potency of ascorbic, gentisic or homogentisic acid. Indeed, on the basis of the linear relationship between L-dopa ingested and L-dopamine excreted in the urine,^{7,8} the quantity of this metabolite excreted would be inadequate to account for the inhibition of the glucose oxidase reduction noted.

When the pH of the urine specimens of patients receiving L-dopa was adjusted to 8.5 with a saturated solution of NaOH and the open containers were allowed

to stand overnight at room temperature, there was a gradual darkening of the urine downward from the surface until the sample was dark brown or black. There was a good correlation between the degree of blackening of the sample and its potency in producing a false negative glucose oxidase reaction.

Since the finding suggested that homogentisic or gentisic acid might be present, representative urine samples producing a false negative glucose oxidase test were analyzed for this material using the thin layer chromatography system described in the previous section on salicylates. In the butanol-water system a single silver reducing spot was noted with an R_f value of 0.86. This corresponded to the spots formed by known homogentisic and gentisic acid. However, by chromatographing extracts of the patient's urine in the 80 per cent saturated ammonium sulfate-2 per cent isopropanol system, both with and without the addition of known gentisic or homogentisic acid, we were able to demonstrate that the intense reducing spot found in the patient's urine (spot X) was neither gentisic nor homogentisic acid (homogentisic acid $R_f = 0.90$ with no fluorescence, gentisic acid $R_f = 0.85$ with fluorescence, compound x, $R_f = 0.80$ with no fluorescence).

A second unidentified spot (spot Y) was consistently present in the ether extract of urine of the patients receiving L-dopa (ammonium sulfate-isopropanol system $R_f = 0.55$ strong fluorescence, and very weakly reduces silver). In an attempt to identify unknown spots X and Y known standards of L-dopa, dopamine, VMA, HVA, ascorbic acid, 5-hydroxyindole acetic acid and tyrosine were chromatographed. However, on the basis of ether solubility, fluorescence, silver reduction and mobility, neither of the unknown spots corresponded to these known compounds.

To ascertain if the reducing compound spot X was capable of affecting the glucose oxidase reaction, this spot was scraped from the plate and extracted with acidified ether. After evaporation of the ether, normal urine adjusted to 1 per cent glucose was added to the residue. Extracts from control areas of the plate were without effect while spot X inhibited the Clinistix glucose oxidase reaction. Urine containing spot X also darkened after addition of sodium hydroxide.

Homogentisic acid was not noted in the urine of patients receiving L-dopa. However, in addition to the usual X and Y spots one patient receiving L-dopa had a spot with both the mobility and fluorescence characteristics of gentisic acid in the absence of known salicylate ingestion.

L-alpha-methyl-dihydroxyphenylalanine (Aldomet), a

TABLE 1

Minimal concentration of L-dopa derivatives that produce a false negative glucose oxidase reaction

Substance	Concentration in urine	Effect
VMA—vanillyl-mandelic acid	15 mg./ml.	none
Homovanillic acid	15 mg./ml.	none
Mandelic acid	10 mg./ml.	none
Phenylalanine	5 mg./ml.	none
Tyrosine	5 mg./ml.	none
L-dopa	2.5 mg./ml.	inhibits
L-dopamine	0.6 mg./ml.	inhibits
Homogentisic acid	0.05 mg./ml.	inhibits

medication used in treatment of hypertension, is structurally related to L-dopa. In high concentrations this medication reduced the Clinitest copper reagent, and in a suspension of 5 mg./ml. preliminary studies indicated inhibition of the glucose oxidase reaction. Five urine specimens obtained from patients taking 1 to 2 gm./day of Aldomet were examined. After adjustment to a 1 per cent glucose concentration, all had a normal glucose oxidase reaction. However, the urine from a patient ingesting 2 gm./day of Aldomet reduced the Clinitest copper reagent. The failure of Aldomet to affect the Clinistix may be due to the fact that it is used in lower doses and/or it is metabolized to different excretory products than is L-dopa.

Serotonin and its metabolites

Five-hydroxyindole acetic acid (5-HIAA) is the main excretory product of serotonin, a biologically potent monamine synthesized from L-tryptophan. 5-HIAA is quite susceptible to oxidation in an alkaline media, reminiscent of the situation noted with homogentisic acid. We, therefore, evaluated the ability of 5-HIAA to influence the Clinistix reaction.

As seen in table 2, 0.25 mg./ml. of this compound can inhibit the glucose oxidase tape, when added to normal urine adjusted to 1 per cent with glucose. Similar results were noted when either the free acid form or the cyclohexyl ammonium salt of this compound was used. L-tryptophan and 5-hydroxytryptophol did not influence the Clinistix reaction, while 5-HIAA and serotonin were less potent in affecting the reaction.

Miscellaneous compounds

The following substances were dissolved in control urine and their effects on the Clinitest, Clinistix and Tes-Tape reaction were noted:

1. Reducing sugars such as galactose, lactose and fructose were studied individually in concentrations of 10 mg./ml. of urine. Although they reduced Clinistix tablets, they did not inhibit the glucose oxidase paper test. Other substances that behaved in this fashion included penicillin G (10,000 U./ml.), streptomycin

(5 mg./ml.), cephaloridine (5 mg./ml.), cephalothin (5 mg./ml.) and isonicotinic hydriazide (INH) (5 mg./ml.).

2. β -phenylpyruvic acid, the substance found in phenylketonuria, affected neither the Clinitest nor Clinistix reaction.

3. Many drugs which are excreted as glucuronic acid conjugates reduce Clinistix tablets. Studies were carried out to see if glucuronic acid itself inhibits the glucose oxidase paper reaction. It was noted that a minimum concentration of 20 mg./ml. was necessary to inhibit the Clinistix reaction and it is unlikely that such quantities of glucuronides are excreted under physiological conditions.

4. Uric acid is one of the natural substances in the urine that tends to inhibit the enzyme tests for glucose.⁹ The related purine xanthine is found in xanthinuria and during allopurinol administration. However, xanthine in concentrations of 2 mg./ml. did not influence the Clinistix reaction test. Caffeine, a commonly ingested xanthine, was also without effect in concentrations of 10 mg./ml. of urine.

Nature of the false negative glucose oxidase reaction

The underlying principle of both the enzyme tapes (Tes-Tape) and sticks (Clinistix) is the air oxidation of urinary glucose catalyzed by glucose oxidase. The reaction produces gluconic acid and hydrogen peroxide and the latter is decomposed by horse radish peroxidase present in the test paper. The nascent oxygen produced converts the oxidation-reduction indicator o-tolidine to its oxidized form, which appears blue. An additional yellow dye such as tartrazine is present in Tes-Tape, increasing the range of colors from pale green to deep blue.

In an earlier report on false negative glucose oxidase reactions Naganna et al. suggested that the interfering chemical inhibited the peroxidase reaction.⁶ Bergemeyer and Bernt have suggested that ascorbic acid interferes with the glucose oxidase method of glucose analysis by competing with the peroxidase for the hydrogen peroxide.⁹ Our data show that all of the interfering substances are potent reducing agents. This suggests that they act by preventing the oxidation of the tolidine indicator, thus influencing the indicator system rather than the enzyme reaction itself. This would make the phenomena of a more general nature, for any reducing substance could produce the false negative reaction. The following experiments support this concept:

1. If the enzyme stick is exposed to a glucose solution until a positive reaction develops and then is im-

TABLE 2

Minimal concentration of serotonin derivatives that produce a false negative glucose oxidase reaction

Chemical	Concentration in urine	Effect
L-tryptophan	5 mg./ml.	none
5-hydroxytryptophol	7.5 mg./ml.	none
5-hydroxytryptamine (serotonin)	1 mg./ml.	inhibits
5-hydroxytryptophan	1 mg./ml.	inhibits
5-hydroxyindole acetic acid	0.25 mg./ml.	inhibits

mersed in a solution of ascorbic, gentisic or homogentisic acid, the indicator color reverts to a negative reading. The minimal concentrations of interfering substance that prevent the color reaction from occurring is adequate to reverse the color after it forms. Such a reversal of color cannot be completely explained by a competition between the interfering agent and peroxidase for the hydrogen peroxide formed.

2. Sodium fluoride is a known inhibitor of peroxidase,¹⁰ but it does not inhibit glucose oxidase.¹¹ When this halide is present in a concentration of 2 mg./ml. in a 1 per cent solution of glucose in urine, it prevents the formation of a positive Clinistix reaction by inhibiting the peroxidase reaction. However, if the color reaction is first allowed to develop in a 1 per cent glucose solution in urine and the strip is then placed in 2 mg./ml. sodium fluoride the color does not fade, for sodium fluoride is not a potent reducing agent.

3. Numerous other reducing agents including cysteine (9 mg./ml.) or glutathione (9 mg./ml.) or sodium bisulfite (5 mg./ml.) were added to urine and could reproduce the phenomenon noted under experiment 1 (above).

When ascorbic acid, 20 mg./ml., was added to normal urine previously adjusted to a 1 per cent glucose concentration, both Clinistix and Tes-Tape gave a negative reaction for glucose. When a lesser concentration of ascorbic acid (10 mg./ml.) was added to the urine, we confirmed the presence of a characteristic type of Tes-Tape reaction.³ The positive reaction occurred above the area where the tape was dipped. The tape acted as a mini-ascending chromatography system, and glucose which probably has a greater R_f than ascorbic acid is present alone at the front, so that a positive reaction can develop. This type of positive reaction was also present in all of the glucose-fortified urine specimens obtained from patients receiving L-dopa or salicylates and those glucose-fortified urine specimens containing homogentisic or 5-hydroxyindole acetic acid. It was also confirmed that if one does not follow the manufacturer's instructions and uses Clinistix like Tes-Tape, dipping only the tip of the test area in the urine rather than completely immersing the area, a line of color develops at the diffusion front. This modification of the manufacturer's instructions also allowed detection of glucosuria in the presence of all of the other reducing agents described in this communication.

DISCUSSION

In the present studies we have demonstrated that homogentisic acid, gentisic acid, 5-hydroxyindole acetic acid and an unknown metabolite of L-dopa can produce

a negative glucose oxidase test in the presence of known glucosuria. The unifying feature of all of these false negative glucose oxidase reactions is the excretion of a potent urinary reducing substance. As our survey of responsible drugs was neither exhaustive nor systematic, one can anticipate that in the future other drugs or diseases may be implicated in the production of this phenomenon.

It is of interest that despite the extensive use of aspirin and the early recognition that it produces false positive Clinistix reaction, a false negative glucose oxidase reaction has not previously been described. This reaction apparently does not occur after casual doses of aspirin. However, with moderate dosage of 2.4 gm. per day, 50 per cent of patients manifested this phenomenon and it becomes more frequent with increasing doses.

It appears that gentisic acid is the reducing agent responsible for this reaction. The percentage of salicylate reported to be excreted as gentisic acid has varied from 1 per cent¹² to 8 per cent.¹³ However, even when present as a minor excretory product, gentisic acid is a potent enough reducing agent to account for the false negative reactions.

This confusing Clinistix reaction should be added to the list of other misleading tests resulting from aspirin ingestion: the false positive Clinistix copper reduction test for glucosuria, and the false positive ferric chloride test for acetoacetic acid.¹⁴

L-dopa is one of the most effective agents found for Parkinson's syndrome, and it will be more widely used in the future.⁸ Although dopamine, a known metabolite of L-dopa, is capable of producing a negative Clinistix reaction in the presence of glucosuria, the concentration required (0.6 mg./ml.) is greater than the amount excreted in patients receiving L-dopa. We believe the responsible agent is an as yet unidentified metabolite compound X. Although it may be structurally related to homogentisic or gentisic acid it is distinct from these compounds in its mobility in the thin layer chromatography systems utilized in these studies.

In a brief report Arras and Balley noted that one patient receiving 16 gm./day of dopa (probably the earlier D-L mixture) had urine and blood levels of homogentisic acid in the range found in alcaptonuria.¹⁵ Cotzias reported that this did not occur when patients received the natural L-isomer in lower doses.¹⁶ He stated that the spontaneous blackening of the urine did not occur after the L-isomer. In contrast, we have found that if the urine is made alkaline it rapidly blackens in many of the patients taking L-dopa. Further studies

are presently in progress in our laboratory on the nature of the reducing substance.

L-dopa has, also, recently been implicated in producing false positive tests for ketone bodies with Labstix (Ames) or Ketostix (Ames).¹⁷ No color formation occurred with Acetest tablet (Ames) test for ketone bodies. Thus on one Labstix, metabolites of L-dopa can produce both a false positive test (ketone bodies) and a false negative test (glucose).

Although we have not had the opportunity to examine the urine of carcinoid patients for a false negative enzyme test for glucose, many of these individuals excrete enough 5-HIAA in twenty-four hours to result in such a test.¹⁸ A related reducing compound, 5-hydroxytryptophan, is excreted in the "atypical carcinoid syndrome." However this compound is not found in a large enough quantity to affect the Clinistix test.¹⁹

The proper use of Tes-Tape for measuring urinary glucose will minimize the false negative reaction. For, when the end of the tape is dipped into the urine and then removed, the liquid front continues to rise on the Tes-Tape and chromatographically separates the glucose from the reducing substances. Thus when the R_f of the glucose and the reducing agent are significantly different, the upper end of the tape may adequately reflect the true glucose concentration. The area of the tape which gives a true reading for urinary glucose may be restricted to a very narrow band and this may make it extremely difficult for the patient to interpret. Clinistix or its modifications (Labstix, Bili-Labstix) will give false negative reactions unless they are used in a modified fashion. These strips must be immersed in the urine so that only the lower part of the glucose indicator region is wet in order that it can act as an ascending chromatographic system and give a true glucose determination. Complete immersion of the test area results in consistently false negative reactions.

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ADDENDUM

Since this manuscript was submitted, Sandler et al. (New Eng. J. Med. 281:1429, 1969) have reported that they could find no homogentisic acid in the urine of patients receiving L-dopa. They identified large quantities of 3, 4-dihydroxyphenylacetic acid (DOPAC). We thus studied DOPAC and found that this compound inhibited the glucose oxidase tape reaction in a con-

centration as low as 0.05 mg./ml. Further thin-layer chromatographic studies of urine from patients receiving L-dopa demonstrated that reducing spot X is indeed DOPAC.

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