# A STUDY TO DETERMINE THE EFFECT OF CONCENTRATED AND DILUTE URINE SPECIMENS ON GLUCOSE URINE TESTING

by

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A FIELD STUDY

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# CHAPTER I

# INTRODUCTION

## Introduction to the Problem

Although the blood glucose concentration is the critical parameter in the assessment of diabetic control, the inconvenience and expense involved in obtaining the measurement justifies the use of urine tests for glucose (Waife, 1973). A number of simple methods for determining glucose content of urine have been developed but each has known drawbacks. Furthermore, additional factors, at present poorly understood, may also affect the accuracy of these methods.

It is the nurse's responsibility to perform the urine tests and interpret the results for hospitalized patients. The nurse who will be administering insulin to the patient should be skilled in performance of these tests, and aware of the various factors that may affect their accuracy. Test errors may lead to inaccurate administration of insulin followed by hypoglycemia.

## Statement of the Problem

In addition to clear documentation of drug effects on urine test

accuracy, the literature indicates that overreading (obtaining a higher glucose reading than the actual glucose level in the urine) may occur in highly dilute urine specimens. The Clinitest method is reported to be the most likely method to over-estimate glucose in dilute second voided urines although overreading may also occur with Clinistix, Diastix and Tes-Tape (Feldman & Lebovitz, 1973).

Urine tests for glucosuria are performed on second voided urine specimens to obtain a more accurate estimation of glucose concentration at a specific time. Random or first voided urine specimens include glucose which has accumulated in the urine since the last time the patient voided. Therefore, the patient is asked to empty his bladder, discard the urine, drink two glasses of water, urinate in one-half hour, and test this second-voided specimen for glucose. The procedure for obtaining a double-voided urine specimen often results in the diabetic undergoing a mild diuresis. The problem is: Does dilute urine resulting from a mild water-induced diuresis affect tests for glucosuria?

## Review of Related Literature

The development of new rapid, simple, modern urine tests have made urine testing easy for patient, physician, and nurse. The tablet, tape and dipstick tests for glucosuria are advantageous because they require no laboratory and can be performed and read

correctly by the physician or nurse in the clinic or hospital as well as by the informed patient at home (Kark, Lawrence, Pollak, Pirani, Muchroke, & Silva, 1963). For all practical purposes they have replaced the classic but cumbersome Benedict's test for routine diabetic urine testing.

Three of these urine tests--Clinitest, Diastix and Tes-Tape will be discussed and their problems and reliability reviewed.

## Clinitest

The Clinitest Reagent Tablet, introduced in 1942, and now used extensively to test urine for glucose, provides a semi-quantitative estimation of sugar in urine and is a reliable, single-tablet, copper reduction test that can detect urine sugar levels of 0.25 per cent (Ackerman, Williams, Packer, Hawkes, & Ahler, 1958; Feldman & Lebovitz, 1973; Kark et al., 1963). Modification of this method can be used to obtain estimations of sugar in excess of 2 per cent and is particularly useful for urine testing in juvenile diabetes (Belmonte, Sarkozy, & Harpur, 1967).

Each Clinitest tablet contains copper sulfate, citric acid, sodium hydroxide and sodium carbonate. Clinitest is a standardized self-heating copper reduction test. Sodium carbonate and a small amount of citric acid facilitate rapid solution of the tablet. Reducing sugars in the urine react with copper sulfate reducing the cupric ions

to cuprous oxide. This reaction results in a color change of blue through green to orange, depending upon the amount of reducing agent present. The sodium hydroxide provides the alkaline medium for the reaction. Sodium hydroxide plus the reaction between sodium hydroxide and citric acid supply sufficient heat for the reaction to take place (Feldman & Lebovitz, 1973; Kark et al., 1963).

Clinitest tablets are stable when stored in unopened bottles at room temperature. The tablets are hygroscopic because they contain anhydrous sodium hydroxide and deteriorate in the presence of moisture. Exposure to heat also causes the tablets to deteriorate. The appearance of the tablets indicates their condition; that is, bluish-white, slightly spotted tablets react satisfactorily while tablets with dark blue dis-coloration in spots or in overall appearance will not give accurate test results (Ames Company, #124870).

Since the Clinitest tablets test for many reducing substances other than glucose, false positives may occur when lactose, galactose, pentoses, homogentisic acid or ascorbic acid are present in the urine in sufficient quantities to reduce the Clinitest tablet. Dahlen found that large dosages of salicylates (60 to 80 grains per day) do at times affect the response to the Clinitest tablet (1971). Ascorbic acid, while a powerful reducing agent, must be present in very large amounts to interfere with Clinitest reactions. This can occur in patients receiving ascorbic acid intravenously or having

a continued high dietary ascorbic acid intake (Free, A. & Free, H., 1970; Hansten & Owyang, 1968). High tissue levels of ascorbic acid, ovulation, or infectious processes such as measles and tuberculosis may lead to sufficient levels of urinary ascorbic acid excretion to cause positive Clinitest results (Morris & Quittner, 1970).

Other medications which may cause false positive tests with Clinitest are: Nalidixic acid (Neg gram ®), Netaxalone (Skelaxin® ), Cephalosporins (Keflin® , Keflex ® , Loridine ® ) and Probenecid (Benemid ® ) (Ames Company, #762 9857).

Glucuronic acid may also give false positives. However, most urinary metabolites containing glucuronic acid are ethers and are not easily decomposed (Morris & Quittner, 1970). The only natural metabolite that gives a false positive consistently is homogentisic acid which appears in the urine in significant amounts only in the condition alkaptonuria (Feldman, Kelley, & Lebovitz, 1970; Morris & Quittner, 1970).

The reliability of the Clinitest method for testing for glycosuria has been studied by several researchers (Ackerman et al., 1958; Cook, M., Free, A., & Giordano, 1958). In the study previously cited (Ackerman et al., 1958) six hundred and sixty urine specimens from 49 juvenile diabetics were tested with Benedict's solution, Clinitest, Tes-Tape and Clinistix. The Benedict's qualitative test and Clinitest appeared somewhat more

reliable in distinguishing glucose levels near 500 milligrams per cent (. 5 per cent) from those higher than 1, 000 milligrams per cent (1 per cent). Clinitest was the only test where a color change clearly indicated a glucose concentration higher than 2, 000 milligrams per cent (2 per cent). Cook, Free, A. and Giordano, using Benedict's solution and the Clinitest method to test six hundred and ninety urines from hospital paitents, found that every urine that tested positive with Clinitest tested positive with Benedict's test (1958). When the study was repeated on healthy subjects the findings were the same (Cook et al., 1958).

Diastix

Diastix Reagent Strips are clear, flexible plastic strips with a pale blue area of reagent-impregnated paper on one end. The reagent area contains the enzymes glucose oxidase and peroxidase. Potassium iodide serves as the indicator and in conjunction with a blue background dye, effects a color change. When Diastix is dipped in urine containing glucose, the enzyme glucose oxidase catalyzes the oxidation of glucose to form gluconic acid and hydrogen peroxide (Ames Company, #124870; Free, A., & Free, H., 1970). A second enzyme peroxidase catalyzes the reaction of hydrogen peroxide with potassium iodide to form free iodine. The iodine blends with the background dye to produce the color change which

indicates the concentration of urinary glucose (Ames Company, #124870).

Diastix provides a semi-quantitative determination of glucosuria. The test is specific for glucose and will react with no other sugar or reducing substance found in urine. Diastix detects glucose concentrations ranging from as little as one-hundredth per cent to one-tenth per cent depending on the amount of inhibitory substances present in the urine (Ames Company, #124870).

Diastix remain useful for approximately two years when stored in a cool, dry place or at room temperature in tightly closed containers. Refrigeration results in unreliable test results. Exposure to direct light, excessive heat (exceeding 86$^{o}$F), and humidity cause the strips to deteriorate. Only Diastix whose reagent area is the same color as the "NEGATIVE" color block on the color chart should be used for urine testing. Any alteration in color may indicate loss of reactivity and such strips cannot be used for accurate testing (Ames Company, #124870).

Since Diastix is dependent upon an enzymatic reaction which is temperature sensitive, urine specimens must be between room temperature and body temperature to provide reliable results. Refrigerated specimens depress the color change. Another source of error occurs in urine specimens with moderate to large amounts of

ketones, since ketones depress the color response of Diastix (Ames Company, #124870).

Diastix are simple to use but strict compliance with testing directions is essential to obtain reliable results. Diastix is designed primarily for at home use by diabetics who are unlikely to have moderate to large ketonuria (Ames Company, #124870).

No research studies on the reliability of Diastix could be found in the literature.

Tes-Tape

Tes-Tape is a strip of paper impregnated with the reagents glucose oxidase, peroxidase and orthotolidine. When Tes-Tape is dipped in urine containing glucose, the enzyme glucose oxidase catalyzes the oxidation of glucose to form gluconic acid and hydrogen peroxide. A second enzyme peroxidase catalyzes the reaction of hydrogen peroxide with orthotolidine to form a blue color (Free, A., & Free, H., 1970).

Like Diastix, Tes-Tape is specific for glucose. It is a simple, easily performed test for glucosuria (Feldman et al., 1970; Haunz, 1964; Weller & Greene, 1966). To obtain reliable results it is recommended that the user of Tes-Tape lay the strip flat against a white background to prevent light from diffusing through the tape and blanching a color response. If the tape shows a 1/2 per cent sugar

concentration or higher, the tester waits one more minute and makes the final comparison (MacNeil, 1961).

A study by Comer using 1500 urine specimens found Tes-Tape to be 96 per cent accurate in glucose concentrations of 0 to 2 per cent (1956). Test results were not influenced by variations in pH and temperature of the urine, or by the presence of common drugs or metabolites in the urine (Comer, 1956). Leonards also studied the enzyme preparation Tes-Tape and found it to be useful for the qualitative detection of glucosuria (1957). Tes-Tape was found to be a satisfactory quantitative method for urines containing 0.25 per cent glucose or less (Leonards, 1957).

Since Comer and Leonards' findings were in disagreement, Bell and Jumper undertook a study to re-evaluate the reliability of Tes-Tape as a quantitative determination of glucosuria (1958). Results showed the accuracy of Tes-Tape to be 94 per cent or higher. Incorrect results in each case were higher than the true glucose level in the tested urine (Bell & Jumper, 1958).

Eli Lilly and Company improved their methods of processing and packaging Tes-Tape in October 1956. Seltzer tested the improved product and found that when used according to manufacturer's instructions, Tes-Tape was a reliable semi-quantitative measure of glucosuria (1958). In a study previously cited Feldman reported that false negative glucose oxidase reactions with

Clinistix occurred with moderate dosages of 2.4 grams of aspirin per day (1970). Fifty per cent of patients receiving this dosage of aspirin manifested the phenomenon. Feldman recommended that in the presence of such reducing agents as aspirin, tests for glucosuria should be carried out with Tes-Tape (1970).

## Responsibility of the Nurse

The nurse is often responsible for collecting the hospitalized diabetic's urine and testing it for glucose, for knowing how to test the urine accurately and safely, and to be aware of false positive or false negative results (Beland, 1970). It is also the nurse's responsibility to know how the urine should be collected and whether to obtain a first or second voided specimen (MacNeil, 1961; Mohammed, 1964). Accurate testing is essential because insulin dosage is frequently regulated according to the quantity of glucose in the urine (Beland, 1970; Belmonte et al., 1967; Dobson, Shaffer, & Burns, 1968; Feldman & Lebovitz, 1973; MacNeil, 1961; Williams, 1971). Strict compliance with the manufacturer's directions is imperative because improper handling or deterioration of the test material may produce inaccurate results. For example, touching the indicator end of Diastix or Tes-Tape or exposing the test material to heat, light or moisture can cause unreliable test results (Mohammed, 1964).

The interpretation of urine test results depends upon the skill with which they are performed and reported, the inherent accuracy of the test, and the patient's renal threshold for glucose (Waife, 1973). In normal conditions, no glucose is excreted in the urine; all that is filtered is reabsorbed. As the plasma concentration of glucose increases above the renal threshold, glucose appears in the urine (Pitts, 1968). In normal individuals the venous blood sugar level exceeds 140 to 190 milligrams per cent before glucosuria occurs (Derr, 1970; Haunz, 1964; Morris & Quittner, 1970).

The effect of various medications on urine tests for glycosuria was included in the discussion of each specific testing material in this study. Several published studies have described the extent of problems caused by these and other factors in a clinical setting (Feldman et al., 1970; Feldman & Lebovitz, 1973; Hansten & Owyang, 1968; Morris & Quittner, 1970). The following section describes the research findings on other sources of inaccurate urine test results. It includes a discussion of the effect of skill in performing the tests and the possible effect of urine concentration on tests for glucosuria.

## Other Sources of Inaccurate Urine Test Results

Dobson, Shaffer and Burns studied the reliability of urine tests for sugar and acetone as performed by hospital nursing

personnel (1968). Urine samples were obtained from a healthy subject during water diuresis. Glucose was added in weighed amounts to exact volumes of urine to obtain a known concentration of glucose. Findings with the 5-drop Clinitest method showed that 12 per cent of the tests were sufficiently inaccurate to result in significant errors in diabetic management. Inaccuracies were attributed to lack of familiarity with the test procedures, personal bias, and deficiencies in the tests themselves (Dobson et al., 1968).

Another researcher, Williams, also investigating the reliability of urine tests for sugar performed by hospital nursing personnel, prepared specimens composed of water, acetone, 50 per cent dextrose in water and food coloring (1971). The Clinitest method was used. The data revealed that significant errors in urine testing were made. The reliability of the tests was impaired both when the subject had knowledge of the patient's previous urine tests and when the subject performed the tests frequently during the week (Williams, 1971).

Feldman and Lebovitz reported the only study found in the literature investigating the possible effect of urine concentration on tests for glucosuria (1973). This question arose from the original study on factors that cause misleading results in tests for glucosuria. Urine specimens were collected from non-diabetic patients and tested for glucose with Tes-Tape, Clinitest, Clinistix and Diastix.

The 389 negative specimens were then fortified to a final glucose concentration of 1/2 per cent and the tests were repeated. Results showed false low readings (a lower glucose reading than the known fortified concentration of glucose in the urine) in 85 of the urines. Twenty-five per cent underread with Diastix; none underread with Clinitest or Tes-Tape. False low readings were often associated with the use of medications such as aspirin, ascorbic acid, digoxin, valium, ampicillin and multivitamins. False high readings occurred even more frequently than false lows. Of 389 specimens, 172 gave false high results. Clinitest tablets overread with 65 per cent of the specimens. Of these, 89 tested at 3/4 per cent and 22 tested at 1 per cent. Many urine specimens testing falsely high were pale in appearance. Their osmolality was usually lower (less than 100 milliosmols per kilogram) than the osmolality of specimens that accurately reflected glucose content (Feldman & Lebovitz, 1973).

It is interesting to note that Dobson, et al., also reported overreading with Clinitest (1968). Screening technicians as well as a majority of ward personnel obtained some readings of 3/4 per cent, an overreading from the actual fortified glucose concentration of 1/2 per cent. The authors attribute the overreading to small differences in drop size and in the colloid concentration of the urine (Dobson et al., 1968).

To determine if a mild water-induced diuresis which occurs

when patients produce a second voided urine specimen could affect tests for glucosuria, Feldman and Lebovitz collected random urine specimens from nine healthy subjects (1973). All specimens tested negative for glucose. Urine osmolality was measured. The specimens were then fortified to a final glucose concentration of 1/2 per cent. Results accurately estimated glucose content in eight of the nine first-voided fortified urine specimens tested with Clinitest. The ninth specimen tested 3/4 per cent glucose. When the fortified second voided specimens were tested with Clinitest, all nine tests overestimated glucose content. Eight showed 3/4 per cent, an overeading from the actual 1/2 per cent glucose concentration. The ninth specimen overread at 1 per cent. Overreading occurred in 44 per cent of the 2nd voided specimens tested with Diastix. The authors attribute the overestimation of glucose to dilute urine (Feldman & Lebovitz, 1973).

The research of Feldman and Lebovitz is the only study found in the literature investigating the problem of mild water-induced diuresis and the effect on urine tests for glucosuria (1973). The sample size was small (9 subjects) and the procedure lacked strict control. That is, random urine samples were used; no attempt was made to control food or fluid intake. Therefore, confirmation and extension of this study are justified.

## Summary

A review of related literature indicates that tests for glucosuria are extensively used, but accurate results are difficult to obtain because of the effects of some medications on urine tests and certain characteristics of the urine specimens.

Studies have shown that Clinitest and Tes-Tape are reliable methods for determining glucosuria when used according to the manufacturer's directions. Diastix has been claimed reliable by the Ames Company. No research studies are available to support this statement.

The literature indicates that the nurse is responsible for collecting and testing urine correctly, and for knowing that false positives or negatives can occur and their causes. The nurse is also responsible for interpreting and recording the tests accurately.

Finally, there has been inadequate treatment in the literature of the problem of dilute urine and its effect on tests for glucosuria.

## Purpose of the Study

The purpose of the study is to test the following hypothesis:

The accuracy of Clinitest and Diastix estimation of glucosuria is affected by the state of hydration of the person from whom the urine specimen is collected.

# CHAPTER II

## METHODOLOGY

The study was pre experimental in nature. The data were collected in the Division of Metabolism laboratories in the Research Building at a University Hospital in a metropolitan area.

The subjects were twenty healthy non-diabetic adult volunteers, medical and nursing students and their spouses as well as two physicians. Potential subjects were informed of the study and the requirements of them for collecting the urine specimens. Persons interested in the study and willing to participate were accepted. Only subjects with a history of kidney disease were excluded from the study. A report form was completed for each subject. A copy of this form is found in Appendix A, p. 48.

Each subject was asked to collect two urine specimens according to the following procedure:

1. The subject was allowed no food or fluids after midnight on the date the specimens were to be collected. No food or fluids were allowed until after the urine samples were collected except as explained in step 4 of the procedure, page 17.

2. The subject was asked to void at 6:00 A. M. upon arising and discard the urine.
3. At 7:00 A. M. the subject was asked to void into a clean specimen container and save all the urine. This specimen was labeled #1. (After glucose fortification this specimen became 1F.)
4. The subject was then asked to drink 480 milliliters of water (2 eight ounce glasses) in as few minutes as possible.
5. At 8:00 A. M. the subject was asked to collect a second urine specimen in a clean container. All this urine was saved. This specimen was labeled #2. (After glucose fortification this specimen became 2F.)
6. The subject was asked to bring both specimen #1 and specimen #2 to the laboratory.

Each subject was provided with a written copy of the directions for collecting urine specimens and any questions regarding the procedure were answered by the researcher. Labeled specimen containers were provided by the laboratory of the study institution.

In the laboratory the following procedure was followed:

1. Each urine specimen was tested for protein with Uristix and the results recorded. Specimens with positive results were discarded.
2. Each urine specimen was tested for glucose with Tes-Tape

and the results recorded. No specimen tested positive.

3. Urine volumes were measured and recorded.
4. Test tubes were labeled with the subject's number and specimen number and placed in the test tube holder.
5. To each test tube, 30 milligrams of glucose were added.
6. Six milliliters of urine from each specimen was added to the corresponding test tubes of glucose using a pipette.
7. The fortified urine specimens were mixed with the vari whirl mixer.
8. Each specimen was tested for glucose three times with Clinitest according to the manufacturer's directions and the results recorded.
9. Each specimen was tested for glucose three times with Diastix according to the manufacturer's directions and the results recorded.

A copy of the steps used in testing the urine is included in Appendix B, p. 50.

To determine if overreading results from dilution with water or from a presently unidentified substance added to urine by diuresis, a third specimen (labeled 3F) was prepared for ten subjects, who produced two to three times or more urine in specimen number two than in specimen number one. This third specimen is comparable to

specimen number two; the difference lies only in the method of dilution. Specimen two was diluted by the body after water ingestion while specimen three was artificially diulted by addition of water to a portion of specimen 1 in the laboratory. The daily excretion of creatinine in the urine is constant and unaffected by changes in urine volume (Page & Culver, 1961). Making the assumption that creatinine and solute excretion is constant with time the following formulas were devised:

1. $\frac{\text{Volume of specimen number two}}{\text{Volume of specimen number one}}$ = A reduced fraction

Example:

Subject 18b

$$\frac{\text{Volume of specimen number two}}{\text{Volume of specimen number one}} = \frac{61}{8} \text{ (Reduced fraction)}$$

(See Table 5, p. 26.)

2. Subtract the denominator (in the example above the denominator is 8) in the reduced fraction above from the numerator (in the example the numerator is 61). The answer obtained (53 in the example) equals the amount of water which will be added to the volume of urine in the denominator (8 milliliters) of the reduced fraction. Thus, the third specimen for subject 18b was prepared by adding

53 milliliters of water to 8 milliliters of urine from his concentrated specimen number one.

Correct dilution of specimen number one will result in small differences in creatinine values between specimens two (2F) and three (3F). These third specimens were fortified with glucose and tested with Clinitest and Diastix three times and the results recorded.

Overreading in third specimens would suggest dilution of urine with water is the factor responsible, while correct readings of 1/2 per cent would suggest that an unidentified substance added to urine by diuresis is responsible for overreading in dilute urines.

Repeated measures were made on urine specimens from five subjects who were willing to collect urine specimens a second time. Finally, all urine specimens were frozen and sent to the Clinical Pathology Laboratory for quantitative glucose and urine creatinine. Glucose concentrations were obtained using the automated hexokinase procedure on the CentrifiChem, an auto analyzer (Union Carbide; Dalal, Cilley & Winsten, 1972; Lustgarten & Wenk, 1972). Creatinines were also measured on the CentrifiChem (Cook, 1971; Fabiny & Ertingshausen, 1971). Creatinines were used as a measure of urine concentration rather than urine osmolalities because they required less laboratory time and expense. All samples were assayed on the same day to eliminate interassay variation.

Urine specimens were collected from 24 subjects. Four subjects were excluded from the study for the following reasons: 1. failure to follow procedure for collecting urine specimens; 2. recent cystitis; 3. proteinuria, and 4. uninterpretable color change with Clinitest tablet.

An analysis of data included the following steps:

1. Data were tabulated to describe the subjects according to age, sex, history of diabetes, etc.
2. Overreading and underreading of fortified samples on tests for glucosuria were described.
3. Pearson's R was used to measure the relationship between the per cent glucose and the creatinine concentration of the fortified urine specimens 1F and 2F (Downie & Heath, 1970). This statistic was computed separately for Clinitest and Diastix first with the original 20 concentrated urines and then with the 20 dilute second voided urines.

# CHAPTER III

# RESULTS

This study describes Clinitest and Diastix results on glucose fortified urine specimens obtained before and after water ingestion. The study population consisted of 20 normal adult volunteers.

## Analysis of Data

### Description of the Sample

The sample consisted of 20 subjects. The average age for all subjects was 30 years with a range of 22 to 40 years. Of the 20 subjects, eleven were female and nine were male. Refer to Table 1 for age distribution.

Table 1. Frequency Distribution of Subjects by Age and Sex.

| | Age Range (Years) | | | | |
|---|---|---|---|---|---|
| Sex | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 |
| Males | 1 | 4 | 3 | 1 | 0 |
| Females | 3 | 3 | 2 | 2 | 1 |
| Total | 4 | 7 | 5 | 3 | 1 |

Five subjects were taking medications regularly at the time the data was collected. See Table 2. Subject number 13 was taking aspirin which has been shown to cause false positive Clinitest and false negative Diastix results. However, he was included in the study because the dosage of aspirin was moderate (45 grains per day) and his concentrated urine specimen (1F) tested appropriately with Clinitest and Diastix. See Tables 2 and 6, p.

Table 2. Distribution of Subjects by Medications.

| Subject number | Medication | Dosage | Frequency |
|---|---|---|---|
| 2 | Premarin | 0.625 mg. | 20 of 30 days |
| 4 | Orthonovum | 1/50 grains | 20 days |
| 11 | Ovral | | 21 days |
| 13 | Aspirin | 15 grains | 3 times daily |
| 20 | Stelazine<br>Tetracycline | 4 milligrams<br>500 milligrams | daily<br>daily |

Six subjects reported a family history of diabetes. Refer to Table 3, p. 24.

A summary of information regarding weight of the subjects indicates 13 were within normal range, one was underweight, and 6 were overweight. None was obese. For the purpose of this study normal weight is defined as ideal weight values as found in the Metropolitan Life Insurance tables (Bogert, Briggs & Calloway, 1966). Overweight is defined as 10-20 per cent above ideal weight

Table 3. List of Subjects and Family Members with Diabetes.

| Subject number | Onset | Family Member(s) |
|---|---|---|
| 6 | Adult | paternal grandmother, paternal uncle |
| 12 | Adult | father, paternal grandmother |
| 13 | | father, brother, paternal grandmother |
| 14 | Adult | maternal great-grandmother |
| 15 | Adult | great grandmother |
| 19 | Adult | mother, maternal grandmother |

Table 4. Distribution of Subjects by Weight and Sex.

| Sex | Under weight | Normal | Over weight | Obese |
|---|---|---|---|---|
| Males | 1 | 6 | 2 | 0 |
| Females | 0 | 7 | 4 | 0 |
| Totals | 1 | 13 | 6 | 0 |

and obesity as 20 per cent or more over ideal weight. See Table 4, p. 24, for the distribution of subjects by weight and sex.

Table 5 contains information regarding urine volumes, creatinines and quantitative glucose values for all subjects. Glucose was weighed in calculated amounts and added to urine specimens to obtain a final known concentration of 0.50 per cent. Assay of glucose concentration by Autoanalyzer revealed actual values ranging from 0.51 to 0.67 per cent glucose. The mean was 0.59 per cent glucose. Refer to Table 5, p. 26.

Verification of correct dilution of specimen number one is observed in the small differences in creatinine values between specimens two (2F) and three (3F). Differences were less than one hundred milligrams per 100 milliliters for all specimens with a range of 1.0 to 91.0 milligrams per 100 milliliters of urine. See Table 5, p. 26 for creatinines.

## Glucose Test Results

After the addition of glucose, one of the 25 urine specimens obtained before water diuresis overread with Clinitest and 8 overread with Diastix. Underreading was observed in 2 of the first urine specimens tested with Clinitest and in 4 tested with Diastix. Twenty-two of first specimens read as 0.5 per cent when tested with Clinitest, while thirteen did with Diastix. See Table 6, p. 27

Table 5. Urine Volumes, Creatinines and Quantitative Glucose For All Subjects.

| Subject number | Volume of specimen #1 in milliliters | Volume of specimen #2 in milliliters | Creatinine milligrams/100 milliliters Specimen 1F | Creatinine milligrams/100 milliliters Specimen 2F | Creatinine milligrams/100 milliliters Specimen 3F | Quant. Glucose milligrams/100 milliliters Specimen 1F | Quant. Glucose milligrams/100 milliliters Specimen 2F | Quant. Glucose milligrams/100 milliliters Specimen 3F |
|---|---|---|---|---|---|---|---|---|
| 1 | 25 | 110 | 211.4 | 60.0 | | 574 | 530 | |
| 2 | 24 | 84 | 62.6 | 40.6 | | 550 | 570 | |
| 3* | 102 | 276 | 37.2 | 12.4 | 14.0 | 574 | 526 | 586 |
| 4a* | 28 | 184 | 180.0 | 35.4 | 30.2 | 632 | 582 | 582 |
| b | 24 | 140 | 194.4 | 41.2 | 36.8 | 618 | 618 | 570 |
| 5 | 30 | 38 | 165.8 | 125.2 | | 604 | 600 | |
| 6 | 85 | 260 | 73.0 | 23.4 | | 556 | 560 | |
| 7* | 24 | 95 | 216.6 | 52.2 | | 582 | 604 | |
| 8a* | 42 | 174 | 105.8 | 26.2 | | 550 | 560 | |
| b | 16 | 152 | 211.0 | 35.8 | 21.2 | 566 | 560 | 646 |
| 9 | 28 | 98 | 85.4 | 17.2 | 26.6 | 600 | 594 | 556 |
| 10a* | 60 | 252 | 90.8 | 27.0 | 22.6 | 600 | 604 | 642 |
| b | 46 | 214 | 116.4 | 31.2 | 25.2 | 622 | 574 | 656 |
| 11 | 70 | 225 | 78.4 | 11.8 | | 612 | 566 | |
| 12a* | 5 | 170 | 207.8 | 99.2 | 7.8 | 604 | 592 | 566 |
| b | 72 | 160 | 147.6 | 71.0 | 96.6 | 606 | 630 | 642 |
| 13 | 40 | 200 | 143.4 | 31.2 | 25.6 | 656 | 570 | 632 |
| 14 | 30 | 58 | 229.4 | 124.0 | | 552 | 578 | |
| 15 | 74 | 290 | 128.8 | 23.4 | 32.6 | 646 | 530 | 542 |
| 16 | 18 | 80 | 285.2 | 93.0 | 77.6 | 636 | 572 | 572 |
| 17* | 74 | 74 | 77.0 | 85.0 | | 512 | 534 | |
| 18a* | 20 | 374 | 215.4 | 26.2 | | 642 | 590 | |
| b | 48 | 366 | 133.4 | 14.2 | 19.8 | 618 | 638 | 672 |
| 19 | 40 | 47 | 231.0 | 134.4 | | 556 | 560 | |
| 20 | 72 | 72 | 169.6 | 129.2 | | 616 | 656 | |

*Subjects with repeated measures.

Table 6. Results of First and Second Specimens on Tests for Glucosuria in Per Cents.

| Subject number | Clinitest 1F | Clinitest 2F | Diastix 1F | Diastix 2F |
|---|---|---|---|---|
| 1 | 1/2 | 3/4 | 1/2 | 1 |
| 2 | 1/2 | 1/2 | 1/2 | 1/2 |
| 3 | 3/4 | 1 | 1 | 1 |
| 4a | 1/2 | 3/4 | 1/2 | 1 |
| b | 1/2 | 3/4 | 1/4 | 1/2 |
| 5 | 1/2 | 1/2 | 1/2 | 1/2 |
| 6 | 1/2 | 1 | 1 | 1 |
| 7 | 1/2 | 3/4 | 1/2 | 1 |
| 8a | 1/2 | 3/4 | 1 | 1 |
| b | 1/2 | 3/4 | 1/4 | 1/4 |
| 9 | 1/2 | 3/4 | 1/2 | 1 |
| 10a | 1/2 | 3/4 | 1 | 1 |
| b | 1/2 | 3/4 | 1 | 1 |
| 11 | 1/2 | 1 | 1 | 1 |
| 12a | 1/4 | 1/2 | 1/2 | 1 |
| b | 1/2 | 1/2 | 1/4 | 1/4 |
| 13 | 1/2 | 3/4 | 1/2 | 1/2 |
| 14 | 1/2 | 1/2 | 1/2 | 1 |
| 15 | 1/2 | 3/4 | 1/2 | 1 |
| 16 | 1/4 | 1/2 | 1/4 | 1/4 |
| 17 | 1/2 | 1/2 | 1 | 1 |
| 18a | 1/2 | 3/4 | 1 | 1 |
| b | 1/2 | 3/4 | 1/2 | 1 |
| 19 | 1/2 | 1/2 | 1/2 | 1 |
| 20 | 1/2 | 1/2 | 1/2 | 1/2 |

for values obtained with Clinitest and Diastix in first urine specimens.

Overreading was observed in sixteen of 25 second urine specimens obtained after water ingestion when tested with Clinitest and in seventeen when tested with Diastix. Underreading was not seen in second specimens tested with Clinitest and only three underread with Diastix. Nine of second specimens tested with Clinitest and five tested with Diastix read as 0.5 per cent. Refer to Table 6, p. 27.

To avoid duplication, only the average of the three testings with Clinitest and Diastix are reported in Tables 6, p. 27 and 7 p. 29. Clinitest gave variable results on 2 specimens; Diastix varied on 5 specimens. When variation occurred, the specimen was tested a fourth time. The mode is reported in this study.

When diluted samples of first urine specimens (3F) were tested, overreading was seen in eleven of the 13 specimens with Clinitest (3F) and seven with Diastix. No specimens underread with Clinitest and only one dilute specimen (3F) underread with Diastix. Two of the diluted specimens tested with Clinitest and five tested with Diastix read as 0.50 per cent. See Table 7, p. 29. These results are similar to those for the specimens obtained after water loading.

Table 7. Comparison of Diluted Urine Specimens with First and Second Specimens on Tests for Glucosuria in Per Cents.

| Subject number | Clinitest 1F | Clinitest 2F | Clinitest 3F | Diastix 1F | Diastix 2F | Diastix 3F |
|---|---|---|---|---|---|---|
| 3 | 3/4 | 1 | 1 | 1 | 1 | 1 |
| 4a | 1/2 | 3/4 | 3/4 | 1/2 | 1 | 1/2 |
| b | 1/2 | 3/4 | 3/4 | 1/4 | 1/2 | 1/2 |
| 8b | 1/2 | 3/4 | 3/4 | 1/4 | 1/4 | 1/4 |
| 9 | 1/2 | 3/4 | 3/4 | 1/2 | 1 | 1/2 |
| 10a | 1/2 | 3/4 | 3/4 | 1 | 1 | 1 |
| b | 1/2 | 3/4 | 3/4 | 1 | 1 | 1 |
| 12a | 1/4 | 1/2 | 1 | 1/2 | 1 | 1 |
| b | 1/2 | 1/2 | 1/2 | 1/4 | 1/4 | 1/2 |
| 13 | 1/2 | 3/4 | 3/4 | 1/2 | 1/2 | 1 |
| 15 | 1/2 | 3/4 | 3/4 | 1/2 | 1 | 1 |
| 16 | 1/4 | 1/2 | 1/2 | 1/4 | 1/4 | 1/2 |
| 18b | 1/2 | 3/4 | 1 | 1/2 | 1 | 1 |

When Clinitest results with specimens one (1F) and three (3F) were compared, it was observed that twelve of the 13 specimens 3F showed a higher glucose concentration than specimens 1F. No specimen showed less glucose in 3F than in 1F when tested with Clinitest. When overreading was observed in second specimens (2F) it was also observed in the diluted third specimens (3F) with Clinitest. Two of the diluted specimens (3F) showed a higher glucose concentration than in 2F with Clinitest. Refer to Table 7.

Seven of the 13 specimens 3F showed a higher glucose concentration than specimens 1F when tested with Diastix. Three of

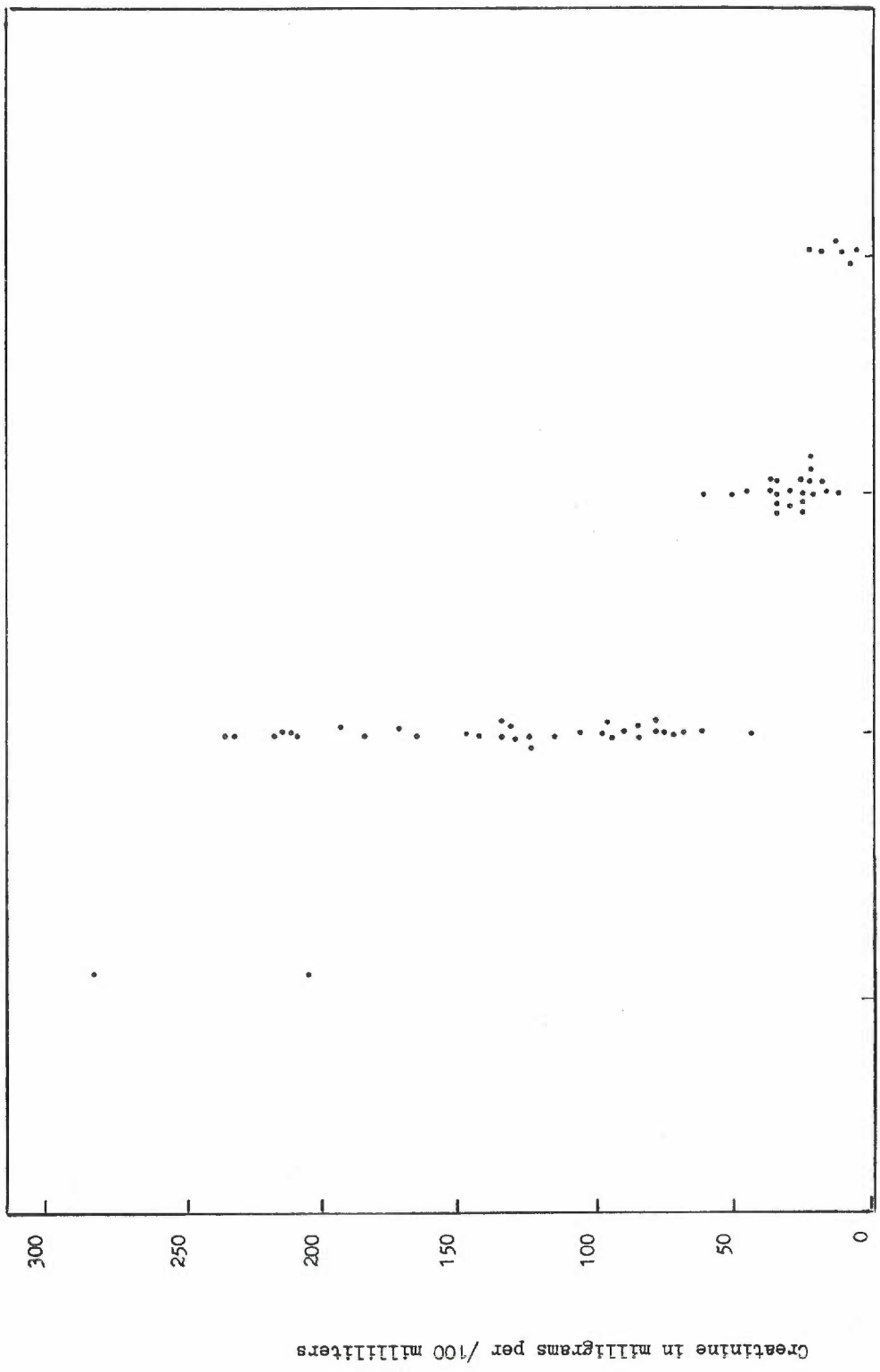
specimens 3F showed a higher glucose concentration than in 2F with Diastix; two showed a lower glucose concentration. These results are reported in Table 7, p. 29. Clinitest and Diastix were not accurate estimators of glucosuria in dilute urine specimens whether the specimens were obtained after water loading or diluted with water in the laboratory. In each case, testing of dilute urine tended to overestimate urine glucose concentration. Refer to Table 7, p. 29.

## Relationship of Creatinine Concentration to Glucose Test Results

Creatinine concentration was compared against the per cent glucose obtained with fortified urine specimens. All specimens which overread with Clinitest had a creatinine concentration of 60 milligrams per 100 milliliters of urine or less. Specimens which read as 1 per cent with Clinitest had creatinines less than 25 milligrams per 100 milliliters. Specimens which reflected the fortified glucose concentration of 0.5 per cent with Clinitest had a creatinine concentration greater than 60 milligrams except for subject two's specimen 2F which had a creatinine concentration of 40.6 milligrams per 100 milliliters of urine. Specimens which underread with Clinitest had a creatinine greater than 200 milligrams per 100 milliliters of urine. Refer to Figure 1, p. 31.

When creatinine concentration versus per cent glucose was

Figure 1. Creatinine Concentration Versus Per Cent Glucose for 63 Fortified Urine Specimens Tested With Clinitest



plotted for Diastix, the relationship was less clear. Refer to Figure 2, p. 33.

Pearson's R was used to assess the relationship between the per cent glucose and the creatinine concentration of the urine specimens. To avoid repetition of very similar results, repeated measures for 5 of the subjects were not included. When Pearson's R was computed for Clinitest with 20 concentrated urines, an r of -.69 was obtained indicating a significant negative relationship between glucose and creatinine values at p of .05 with 19 degrees of freedom. A slightly lower though still significant r (-.62) was obtained with concentrated urines for Diastix at the same alpha level and degrees of freedom.

When glucose and creatinine values for only those 20 urine samples obtained after water loading were used to compute Pearson's R, a high coefficient and negative relationship was obtained for Clinitest; r equals -.84 (p .05 and 19 degrees of freedom). With Diastix an r of -.34 was obtained which was not significant at p of .05 and 19 degrees of freedom.

These findings were interpreted to mean that there is a significant negative relationship between creatinine and glucose values in concentrated urine specimens with both Clinitest and Diastix, and in dilute urine specimens tested with Clinitest. Thus, as creatinine concentration increases--glucose decreases, and the

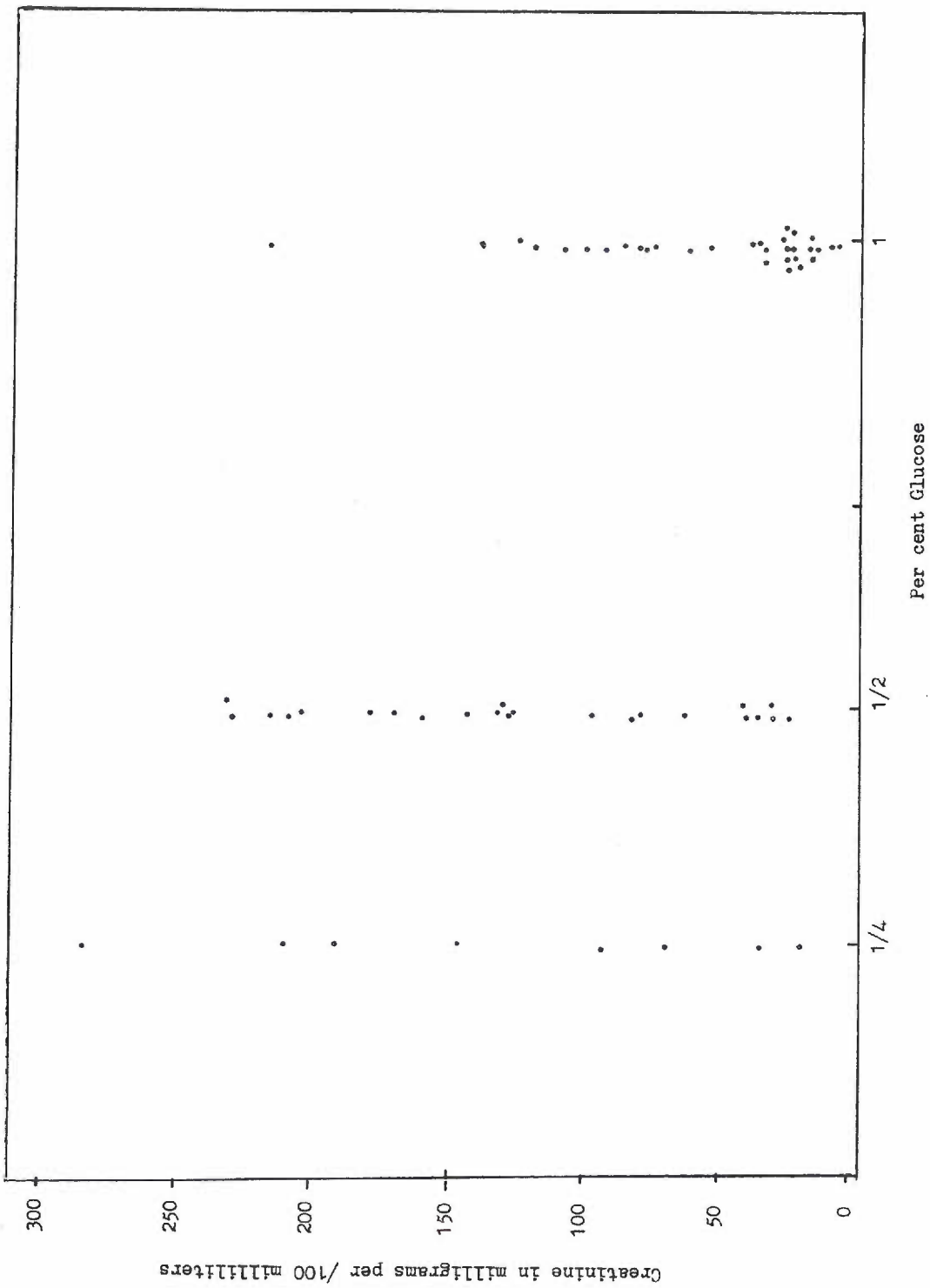



Figure 2. Creatinine Concentration Versus Per Cent Glucose for 63 Fortified Urine Specimens Tested With Diastix

reverse. Glucose values in dilute urines (2F) tested with Diastix did not produce a significant negative relationship.

# CHAPTER IV

## DISCUSSION

Interpretation of the results of this study is complicated by a methodological problem. The researcher planned to use a known final glucose concentration of 0.50 per cent, but laboratory verification of quantitative glucose yielded an actual mean glucose of 0.59 percent. While the source of error cannot be positively identified, three possible sources are: 1. inaccurate weighing of glucose by the researcher, 2. inaccurate standardization of samples by the laboratory, and/or 3. malfunction of the autoanalyzer. While this slightly high glucose level may be cause to question the results of this study, it is interesting to note that glucose values ranging from .60 to .65 per cent yielded glucose test results of .50 per cent with Clinitest and Diastix in concentrated urines and urine specimens obtained after water loading. Refer to Tables 5, p. 26 and 6, p. 27 subject 5 and 20. It must also be pointed out that these two subjects did not diurese with water loading. Where appreciable diuresis occurred--that is, a urine volume of at least two to three times as much for urine specimens obtained after water loading than after water restriction--concentrated first specimens tested .50 per cent with Clinitest and Diastix for subjects 1, 4a, 7, 9, 15 and 18a with

quantitative glucose values of 0.57 to 0.64 per cent. Specimens obtained after water loading from the same subjects tested as 3/4 per cent with Clinitest and 1 per cent with Diastix at lower quantitative glucose values of .53 to .60 per cent. See Table 5, p. 26 and 6, p. 27. Thus, this methodologic artifact not withstanding, the data do seem to indicate that the concentration of urine specimens affects Clinitest and Diastix Test results. Both Clinitest and Diastix more frequently read as .5 per cent with concentrated first specimens than with urine specimens obtained after water loading. Accurate estimation of glucose in concentrated urines occurred more frequently with Clinitest than with Diastix. This finding supports the research of Feldman and Lebovitz (1973). Underreading occurred more frequently in concentrated first specimens with Clinitest than overreading. Feldman and Lebovitz reported no underreading with Clinitest (1973). Overreading was seen more frequently with Diastix than underreading in concentrated first specimens. Over and underreading occurred equally with Diastix in a study by Feldman and Lebovtiz (1973).

Accurate estimation of glucose in dilute second specimens was seen in only nine of Clinitest measurements and five of Diastix measurements. In these specimens, overreading was seen more frequently than underreading with both Clinitest and Diastix. No specimens underread with Clinitest and only three underread with

Diastix. Overreading was observed almost equally with Clinitest and Diastix in urine specimens obtained after water loading. This finding is in opposition to that of Feldman and Lebovitz who found Clinitest the most likely to overestimate glucose content in dilute urines (1973). As Feldmanand Lebovitz reported, significant overestimation of urinary glucose content can occur in dilute urine specimens obtained after water ingestion (1973).

This study supports the judgment of Feldman and Lebovitz that dilute urine seemed to be the significant factor in overestimation of glucosuria. Evidence for this statement is found in the following observations:

1. Urine tests of concentrated first specimens more accurately reflected the actual glucose concentration than did tests of dilute urines.
2. When subjects did not diurese appreciably no overreading occurred in second specimens with Clinitest and tended not to occur with Diastix.
3. When overreading occurred in second specimens obtained after water ingestion with Clinitest it also occurred in corresponding diluted third specimens. Variable results were seen with Diastix.
4. There was a significant negative relationship between creatinine concentrations and per cent glucose with

Clinitest. Specimens which overread with Clinitest had a creatinine concentration of 60 milligrams or less per 100 milliliters of urine.

An effort was made to determine why dilute urine is subject to overreading by diluting a portion of concentrated urines with water. Accurate estimation of glucosuria was seen in only two of the 13 specimens so diluted with Clinitest, and five with Diastix. Overreading was seen in eleven with Clinitest and seven with Diastix. No specimens underread with Clinitest and only one underread with Diastix. These findings suggest that dilution with water and not some substance added to urine by diuresis is the factor responsible for overreading in dilute urine.

The correlation of creatinine concentration with Clinitest results leads to the question of what inhibitor is being diluted out of these urine specimens. Lack of such clear correlation of creatinine concentration with Diastix results suggests that there are other unknown factors involved. Additional studies are needed to investigate these questions.

Finally, physicians and nurses may encourage overestimation of glucose by instructing diabetic patients in the second voided urine procedure. Modification of the technique may help avoid overestimation of glucosuria. Additional studies are necessary to determine if reducing the amount of water the patient ingests before

obtaining a second voided urine specimen whould result in accurate estimation of glucosuria. However, it seems feasible that care in avoiding overly concentrated or dilute urines should increase the accuracy of both Clinitest and Diastix estimation of urine glucose.

# CHAPTER V

# SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

## Summary

Urine testing for glucosuria is part of the daily routine in diabetic management. Several factors have been described which can affect the accuracy of urine tests. The purpose of this study was to test the following hypothesis concerning another possible source of variation in urine test results; The accuracy of Clinitest and Diastix estimation of glucosuria is affected by the state of hydration of the person from whom the urine sample is collected.

The method of investigation was pre experimental. Morning urine specimens were obtained from non-diabetic subjects before and after water ingestion. After checking for the presence of glucose and protein, all specimens were fortified to a known glucose concentration of 0. 59 per cent and tested with Clinitest and Diastix. Concentrated first urine specimens from ten of the subjects were diluted with water to volumes equivalent to those of the specimens obtained after water loading. These specimens were also fortified with glucose and tested. Repeated measurements were made for five subjects. All urine specimens were frozen and sent to the lab

for quantitative glucose and creatinine concentration.

The subjects were 20 adult non-diabetic volunteers, 11 females and 9 males. The mean age was 30 years. Six subjects had a family history of diabetes and five were taking medications regularly.

The results indicated that significant overestimation of glucose content can occur in urine specimens obtained after water ingestion. Overreading was observed almost equally with Clinitest and Diastix in dilute second urine specimens. Dilute urine seemed to be the significant factor in overestimation of glucosuria. There was a significant negative relationship between creatinine concentrations and per cent glucose with Clinitest in concentrated and dilute urines. A significant negative relationship was seen only in concentrated urines with Diastix.

## Conclusions

On the basis of this study in this limited sample only, the following conclusions can be made.

1. The accuracy of both Clinitest and Diastix is affected by the state of hydration of the person from whom the urine sample is collected.
2. Both Clinitest and Diastix tend to overread glucose content in dilute urine specimens whether specimens are obtained

after water loading or by laboratory dilution after water restriction.

3. The concentration of one or more substances present in the urine appears to be the factor affecting the tests, since dilution of concentrated urines with water reproduced the effect of diuresis.
4. Clinitest more often accurately reflected urine glucose concentration than Diastix.

## Recommendations for Further Study

Based on the findings of this study the following recommendations are made.

1. Repeat the study using a larger, random sample and decreasing the water ingested to 8 ounces.
2. Repeat the study using diabetic subjects.
3. Fortify urine specimens with glucose in the amounts of 1 per cent and 2 per cent, and test with Clinitest and Diastix to determine if overreading occurs with marked glucosuria.
4. Attempt to define the inhibitory substance(s) in urine by performing Clinitest and Diastix measurements on glucose solutions with purified components of normal urine added individually.

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# REFERENCES


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# APPENDICES

# APPENDIX A

Report Form

# REPORT FORM

Name _____ Date _____

Age _____ Sex _____

Height _____ Weight _____

Medications _____

Dosage _____

Frequency _____

Family history of diabetes _____

_____

_____

Females (premenapausal) only -
Record day in menstrual cycle _____

Basal Test Results:

| Specimen # | Protein | Tes-Tape |
|---|---|---|
| 1 | | |
| 2 | | |

# APPENDIX B

## Steps Used in Testing Urine

# APPENDIX B

## Steps Used in Testing Urine

1. Test urine with Uristix to check for the presence of protein:
   a. Dip reagent end of Uristix in urine.
   b. Remove immediately.
   c. Tap edge of strip against container to remove excess urine.
   d. Compare reagent area with color chart on Uristix bottle.
   e. Record findings.
2. Test urine with Tes-Tape to check for the presence of glucose:
   a. Tear off strip of Tes-Tape about one and one-half inches long.
   b. Dip one end of tape into the urine.
   c. Remove immediately, place against a white background and wait one minute.
   d. Compare the darkest area with color chart on Tes-Tape dispenser.
   e. Record findings.
   f. If Tes-Tape is negative for glucose, test fortified urine sample with Clinitest and Diastix.
3. Test urine with Clinitest Tablet:
   a. Place 5 drops of urine in a clean, dry test tube holding dropper in upright position.
   b. Rinse dropper with clean water.
   c. Add 10 drops of water to the same test tube.
   d. Drop one Clinitest Tablet in same test tube.
   f. Wait 15 seconds after solution stops boiling.
   g. Shake test tube gently.

h. Compare with color chart accompanying Clinitest tablets.

i. Record findings.

j. Repeat entire procedure 2 more times.

4. Test urine with Diastix:

a. Dip reagent end of Diastix in urine.

b. Wait two seconds and remove.

c. Tap edge of strip against side of urine container to remove excess urine.

d. Wait exactly 30 seconds after removing Diastix from urine.

e. Compare reagent side of Diastix to the closest matching color on the Diastix bottle.

f. Record findings.

g. Repeat entire procedure 2 more times.

AN ABSTRACT OF THE FIELD STUDY OF

BEVERLY J. EPENETER

For the: MASTER OF NURSING

Date of receiving this degree: June 13, 1975

Title: A STUDY TO DETERMINE THE EFFECT OF CONCENTRATED AND DILUTE URINE SPECIMENS ON GLUCOSE URINE TESTING,

Approved: _____
Marie Berger, M. S. Field Study Advisor

The purpose of this study was to determine the effect of the concentration of urine on tests for glucosuria.

Urine specimens were obtained from 20 normal subjects before and after water ingestion. After checking for the presence of glucose and protein all specimens were fortified to a known glucose concentration of 0. 59 per cent and tested with Clinitest and Diastix.

Conclusions

This study was conducted with a limited number of volunteer subjects who were not randomly selected. The findings cannot be generalized to any group other than the one studied.

The results indicate that significant overestimation of glucose content can occur in dilute urine specimens obtained after water ingestion. Overreading was observed almost equally with Clinitest

and Diastix. There was a significant negative relationship between creatinine concentrations and per cent glucose with Clinitest. A significant negative relationship was also seen in concentrated urine specimens with Diastix.