NATIONAL CENTER For HEALTH STATISTICS | Series 2 Number 3

VITAL and HEALTH STATISTICS
DATA EVALUATION AND METHODS RESEARCH

The One-Hour Oral Glucose Tolerance Test

Response of middle-aged men to 100-gram and 50-gram doses of glucose given fasting and 1, 2, and 3 hours after meal.

DHEW Publication No. (HRA) 74-1271

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Health Resources Administration
National Center for Health Statistics
Rockville, Maryland

Vital and Health Statistics-Series 2, No. 3
Reprinted as DHEW Publication No. (HRA) 74-1271
August 1973

First issued in the Public Health Service Publication Series 1000, July 1963

For sale by the Superintendent of Documents, U.S. Government Printing Office,
Washington, D.C., 20402-Price 30 cents

PREFACE

This is one of several studies designed to evaluate examination procedures used in the first cycle of the Health Examination Survey. These studies constitute efforts to define and to measure some of the components of variation in the examination techniques. Where the techniques used in the Health Examination differ from standard techniques, an effort is made to evaluate the differences. This report deals with the glucose tolerance test.

In order to accommodate a glucose tolerance test within the Health Examination Survey it was necessary to modify the standard glucose tolerance test in a number of respects. Where the standard test requires at a minimum that the person come in for examination after an overnight fast, drink 100 grams of glucose, and submit to several venipunctures, the Health Examination used a test which required no advance preparation, a challenge of 50 grams of glucose, and only one venous specimen. While expert advice had suggested that this modified test would yield meaningful data on glucose tolerance, it seemed desirable to evaluate this test more precisely by a special study.

Similar problems are faced by a number of ongoing epidemiological studies in which diabetes and its relationships to other diseases are being examined. One such investigation, the Tecumseh Community Health Study, carried out under the auspices of the Center for Research in Diseases of the Heart, Circulation and Related Disorders of the University of Michigan, supported by Grants H-4145 and HE-06378 from the National Heart Institute, the National Institutes of Health, Bethesda, Maryland, is using a similar test procedure employing a load of 100 rather than 50 grams of glucose. A collaborative study to evaluate these procedures was set up under the direction of Dr. Norman S. Hayner, a member of the Center Research Staff. Appreciation is expressed to Dr. Thomas Francis, Jr., Director of the Center, and to other members of the Tecumseh Study Staff, particularly Dr. Frederick H. Epstein, Dr. Marcus O. Kjelsberg, Dr. Benjamin C. Johnson, and Dr. Millicent W. Payne for advice on certain aspects of the project.

The study was conducted in the Federal Correctional Institution at Milan, Michigan. For assisting in many ways we owe thanks to Mr. L.B. Stevens, Warden, to Dr. Roland Ware, Chief Medical Officer, and to members of their staffs. The volunteers who submitted to the long and uncomfortable series of test procedures deserve special credit.

Field tests were performed under the supervision of Dr. Hayner by Mr. Alberto Faustino and Mr. Keith Lepard. Food records were translated into estimates of carbohydrate intake by Mrs. John Vandenbelt, Research Dietitian with the Tecumseh Study.

Laboratory work was done by the Diabetes Field Research Unit of the Diabetes and Arthritis Branch, Division of Chronic Diseases, Bureau of State Services, U.S. Public Health Service. For this and for his technical advice we would like to thank Dr. John B. O'Sullivan, Director.

For the special studies which are carried out at its expense but are not directly conducted by the National Health Survey Division, staff members are assigned for liaison with the research organization doing the study. Dr. Alice M. Waterhouse and Mr. Tavia Gordon participated in the design of the study and kept closely informed on the study progress, conveying the viewpoint of the National Health Survey Division on questions of methodology.

CONTENTS

SYMBOLS

Data not available	---
Category not applicable	...
Quantity zero	-
Quantity more than 0 but less than 0.05	0.0
Figure does not meet standards of reliability or precision	*

THE ONE-HOUR ORAL GLUCOSE TOLERANCE TEST

INTRODUCTION

The oral glucose tolerance test is one of the best established diagnostic procedures in clinical medicine. For over 40 years the general form of the test has remained remarkably fixed: the patient fasts overnight and drinks a glucose solution in the morning; before the glucose drink and at intervals during the next 3 or 4 hours blood and urine specimens are obtained for measurement of glucose concentration. Provided the patient is in caloric balance and has been eating sufficient carbohydrate (or has followed a preparatory diet) high blood glucose levels after challenge usually indicate diabetes. In clinical practice the oral glucose tolerance test constitutes the decisive diagnostic test for diabetes.[1, 2]

For large population studies, however, the standard glucose tolerance test is clearly impractical. Generally speaking, participants in such surveys cannot be expected to come to the examination in a fasting state, nor to submit to a procedure lasting several hours and requiring a succession of venipunctures. Hence, surveys are obliged to use some reasonable modification of the standard procedure, shortening and simplifying it, if they are to measure glucose tolerance at all.

Accordingly, the U.S. National Health Survey[3] and the Tecumseh Community Health Study[4]—both of which schedule participants for examination at whatever times of day are most convenient—employ the following procedure: Soon after reporting for examination the participant is given a glucose load (50 grams in the Health Examination Survey, 100 grams in the Tecumseh Study). Venous blood is collected 1 hour later and part of it is preserved for later determination of the blood glucose concentration. Urine is collected within 30 minutes after venipuncture. The same specimens are obtained from diabetics, although known diabetics are not given the glucose drink.

The present study examines two of the variables in these procedures which may alter glucose response from what is obtained in a standard fasting test—the interval from the last meal to the start of the test and the dose of glucose given in the drink.

THE STUDY DESIGN AND SELECTION OF SUBJECTS

The basic design was to submit a small group of individuals to a series of glucose challenges at weekly intervals, varying the glucose dose and the interval between meal and challenge. The size and scope of the study were limited primarily by the number of specimens which could be collected and tested each week. So far as practicable, variables not being studied were controlled. There were two dose levels (50 and 100 grams) and four intervals after meal (1 hour, 2 hours, 3 hours, and overnight). Each individual in the study group was tested twice by each procedure. His average response to one procedure could be compared with his average response to any other procedure. The difference in response to replicates of the same procedure would constitute the measure of his response variability.

This report was prepared by Dr. Norman S. Hayner of the Department of Epidemiology, University of Michigan School of Public Health, and Dr. Alice M. Waterhouse and Tavia Gordon of the U.S. National Health Survey staff.

Procedures were scheduled in a Latin square so that any drift in laboratory determination, seasonal change or conditioning effect appeared as part of the general variability rather than being attributed to the variables under study. All procedures were evaluated for each person and the comparisons for each person pooled for all persons.

The subjects of this study were volunteers from middle-aged male prisoners in the Federal Correctional Institution at Milan, Michigan. About one-third of them were Negro. Known diabetics were excluded. All appeared to be in good general health and were working 40 hours a week in various parts of the institution or in an adjoining industry and farm. As recorded in detailed table VII, only 2 of the 24 men selected for the main study gained or lost more than 4 pounds during the study. Clinical examinations of this group during the study confirmed the impression of good health in most instances. None of these 24 volunteers presented symptoms or signs of pathologic entities known to impair carbohydrate tolerance. Subjects 01 and 21 were the only Phase III participants with a family history of diabetes, the former in a cousin, the latter in his father.

The study was divided into four phases. Phase I consisted of a screening test for each volunteer. Phase II was preliminary evaluation of response variability, which required 2 weeks. Phase III was the main study, lasting 16 weeks. Phase IV, a postlude, was used to help characterize the study group by applying other procedures for testing glucose tolerance.

Phase I

In order to prepare a roster for the study group and to become familiarized with the working conditions and the study techniques, the staff invited all prisoners 40 to 54 years of age to volunteer for an initial screening session. There were, in fact, no volunteers over 53 and there was one 39 years of age; 45 men volunteered. Two to four hours after breakfast, each volunteer was given a drink of 100 grams of glucose. As in the remainder of the study all administered glucose was supplied cold in a 50-percent aqueous solution by weight, with a flavoring of lemon juice at the option of the subject. A blood specimen was taken just before the drink and 1 hour later, and a urine specimen was obtained about 30 minutes after the second blood specimen. The time since breakfast and its content were recorded, but no effort was made to control these factors. The carbohydrate content of the breakfast was estimated in accord with standard tables.[5,6,7] Half of the group was done one week, half the next. The results are recorded in detailed table I.

In addition to providing experience in techniques and procedures, this initial phase was meant to serve other important purposes. It gave each volunteer a realistic notion of what the main study required of him. At the same time, it allowed the staff to judge the volunteers and to exclude any men who were unrecognized frank diabetics, had veins that were difficult to enter, or were in some other fashion unsatisfactory subjects for the main study. It also provided an initial gauge of response to glucose challenge, and since there is especial interest in persons with elevated blood glucose values after challenge, this gauge was used in subsequent phases to sample such persons at a higher rate than persons with lower glucose values. Naturally, this procedure would prove effective only if a single blood glucose value provided a fairly reliable measure of a person's usual response. The extent to which this is true will be discussed later. Unfortunately, the selective process was not as effective as hoped because of the limited number of prisoners available in the specified age range.

Phase II

Before initiating the main study, it was felt desirable to undertake two 3-hour glucose tolerance tests on a small group of men. This would provide experience in conducting the standard glucose tolerance test within the restrictions of space and under the special restraints required by the prison. More important, it would measure the variability inherent in response to glucose. Conceivably, the experience obtained during Phase II might indicate that it was desirable to modify the study design.

Accordingly, 10 persons were given a 3-hour oral 100-gram fasting glucose tolerance test. Because the institutional fare was high in carbohydrate and caloric content, no attempt was made to modify the diet of the study participants.

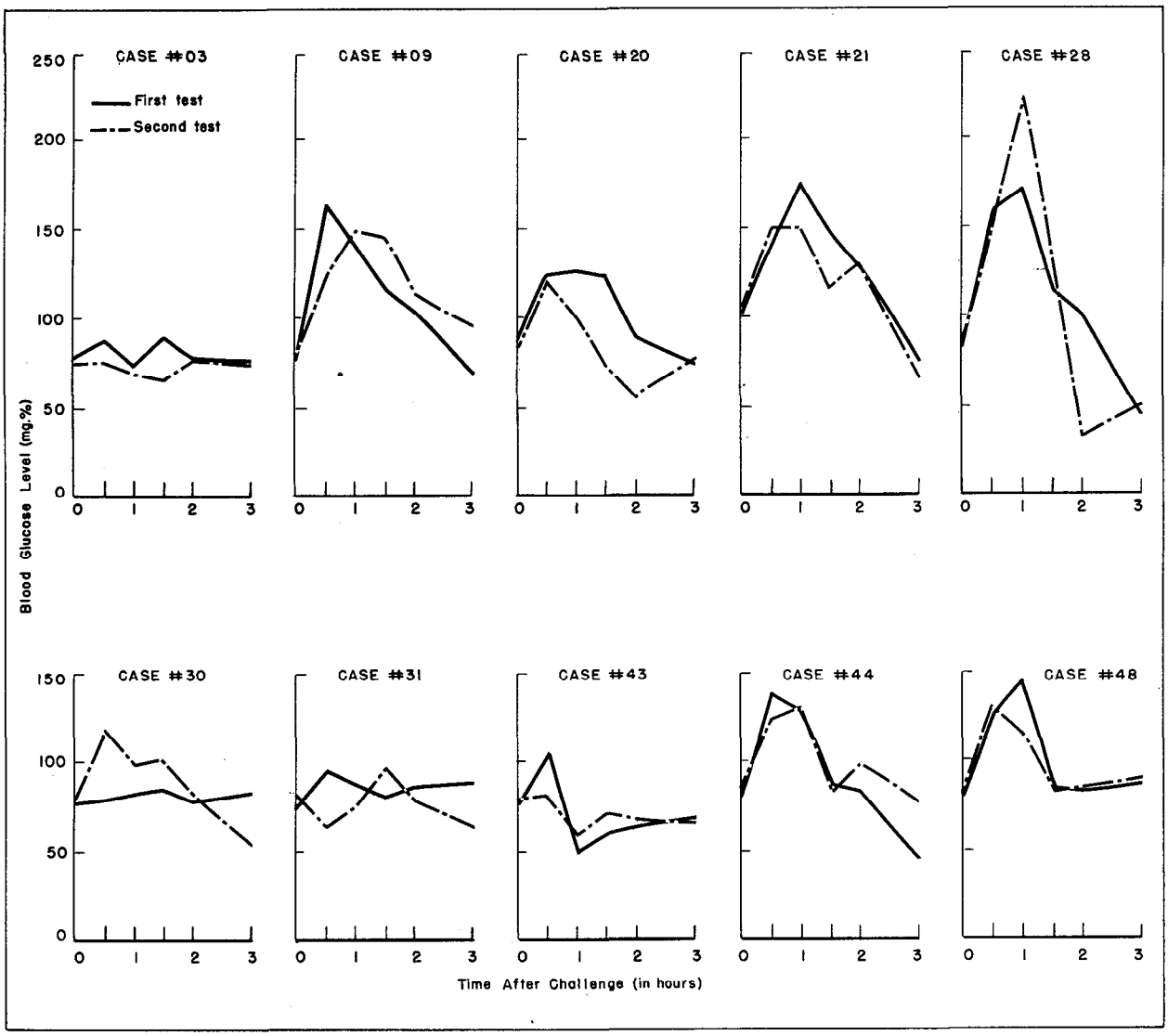

Figure 1. Response to the standard glucose tolerance test, 10 men, Milan, Phase II.

The findings from this phase of the study are indicated in detailed table IV and figure 1. While they are consistent with the limited data already in the literature, it must be admitted that it was startling to see them. It was clear that with the resources available, stable estimates of the amount of difference associated with different procedures could not be obtained, even if the specific group under study were a true population sample, but that it might be possible to establish the existence and direction of such differences. It was decided to continue with the original program of study.

Phase III

The final study group consisted of 24 individuals. The following eight procedures were under study:

A. Overnight fast, challenge of 100 grams
B. Overnight fast, challenge of 50 grams
C. One hour after meal, challenge of 100 grams
D. One hour after meal, challenge of 50 grams
E. Two hours after meal, challenge of 100 grams

F. Two hours after meal, challenge of 50 grams

G. Three hours after meal, challenge of 100 grams

H. Three hours after meal, challenge of 50 grams

In procedure A blood specimens were taken before challenge, and at ½, 1, 1½, 2, and 3 hours after challenge. In the other procedures, blood specimens were taken just before challenge and 1 hour after challenge. Urine specimens were taken about 90 minutes after challenge. Again, the institutional fare was not altered. As in Phase I, all food eaten on the day of the test was recorded and its carbohydrate content estimated.

The volunteers were ranked according to their Phase I blood glucose level 1 hour after challenge. All persons with blood glucose levels at or above 110 mg.% 1 hour after challenge were selected for Phase III (fig.2). In the end, 15 participants came from this group and 9 came from the group with 1-hour blood glucose levels less than 110 mg.% in Phase I.

The 24 participants were placed in 8 groups. Each group consisted of one person chosen at random from the high end of the scale, one from the middle and one from the low. The group was then assigned at random to one of the eight procedures. This initial assignment determined the order in which these three persons moved through the succession of procedures. The final assignments are shown in detailed table II. Since each procedure was duplicated, each person was to be challenged 16 times during Phase III. With only minor exceptions, challenges were given at weekly intervals.

During all of Phase III only 1 episode of vomiting was noted within the 24 hours after glucose administration. Subject number 46 had this experience 6 hours after a 50-gram dose. However, he had had bouts of epigastric discomfort and vomiting for years. Indeed, his fifteenth procedure was deferred because such an episode had begun on the preceding day.

There were several lapses in the execution of this design. In spite of an effort to solicit only men who would be expected to remain at Milan for the entire period of the study, 2 of the original 24 participants were transferred and 1 was paroled before completing the full series of Phase III. The two transferred men were replaced at random from the remaining volunteers. Several men were

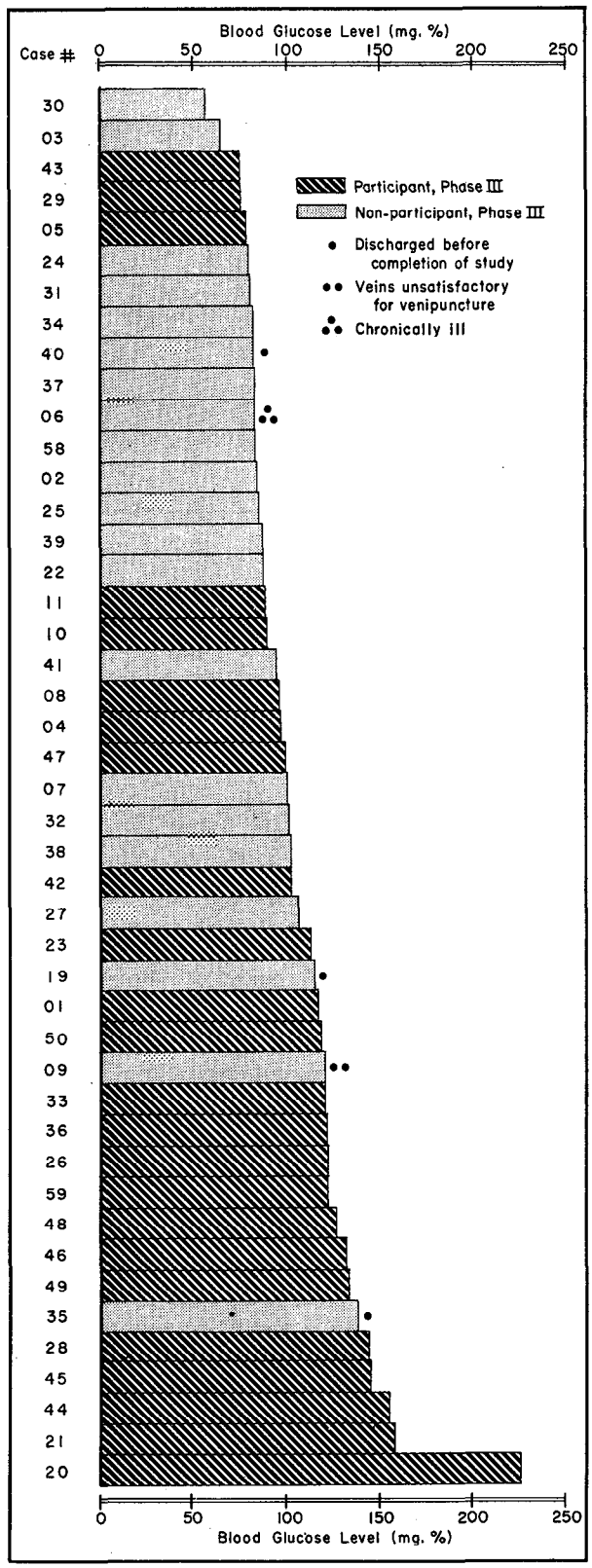

Figure 2. Blood glucose levels 1 hour after challenge, 45 men, Milan, Phase I.

given the wrong dosage one week. The correct dosage was administered to these men after completion of the remaining scheduled tests. Almost all specimens were obtained precisely as intended, but occasionally the time between meal and challenge was different from that planned. Whenever a significant lapse was noted (see detailed table II), an effort was made to supply the correct procedure at a later time during Phase IV.

Phase III started January 24 and ended May 8, 1962. Individual test findings are recorded in detailed table III.

Phase IV

The final 6 weeks afforded time and laboratory support to perform only three fasting tests on each of the remaining 22 participants in Phase III. It was decided to use the following three procedures, each after 3 days of at least 250-300 grams of carbohydrate daily:

1. A standard 100-gram oral glucose tolerance test (SGTT).
2. A cortisone glucose tolerance test (CGTT). A uniform procedure was adopted whereby a dose of 62½ mgs. of cortisone was administered 8½ to 9 hours and again 2½ to 3 hours before a morning fasting challenge of 100 grams of glucose. In contrast, the original procedure of Fajans and Conn[8] would have called for 50 mg. doses instead of 62½ mg. doses of cortisone for individuals under 160 pounds in weight (subjects 21, 29 and 48) and the glucose dose would have been 1.75 grams per kilogram of "ideal body weight".
3. The prednisone glycosuria test (PGT) as described by Joplin, Fraser, and Keeley[9] followed directly by another 100-gram glucose tolerance procedure.

The mean SGTT values were to be compared with means from Phase II and Phase III (procedure A) to check the hypothesis that there should be no difference; i.e., that added carbohydate had been unnecessary. The CGTT and PGT were included to explore their potential applicability and usefulness for population studies. The CGTT would also provide another means for clinical classification of carbohydrate tolerance. It appeared infeasible to perform an intravenous glucose tolerance test.

Laboratory Methods

Blood specimens were shipped on water ice from Milan at the end of each day of tests to the Diabetes Field Research Unit in Brighton, Massachusetts, for determination of glucose concentration by the Somogyi-Nelson Method.[10] A review of technical variability encountered during the study (Appendix I) supports a conclusion that the work of this laboratory was reliable and consistent from week to week and that shipment did not significantly alter the results. Urine samples were tested by the field staff at Milan with a glucose oxidase impregnated tape ("Tes-Tape" produced by Eli Lilly Company, Indianapolis).[11] Quantitative urinalyses during Phase IV were done in Brighton by the Froesch and Renold method.[12]

BLOOD GLUCOSE LEVEL 1 HOUR AFTER CHALLENGE

The primary purpose of the study was to see how blood glucose levels 1 hour after challenge were influenced by differences in the amount and time of glucose challenge. One method of evaluating this is the comparison of mean 1-hour blood glucose levels in response to each procedure, averaged for all 24 persons in the main study (Phase III). The mean value for each procedure is shown in table 1 and figure 3. With table 2, which gives the standard deviations of response, the means reveal several of the major findings.

As expected, the response to a 100-gram oral glucose load was greater than to 50 grams. The difference between the mean of all 100-gram and of all 50-gram procedures combined was 9.4 mg.%. If the mean levels for individuals at different times of challenge are considered, there are altogether 96 comparisons of a 50-gram with a 100-gram challenge. The level was greater after a 100-gram challenge in 65 of these 96 comparisons. In other words, in the majority of instances the 100-gram challenge leads to higher 1-hour blood glucose levels than the 50-gram.

Futhermore, the mean 1-hour level was higher with a challenge of 100 grams than with a challenge of 50 grams, whether the glucose load was given to a fasting individual or was given

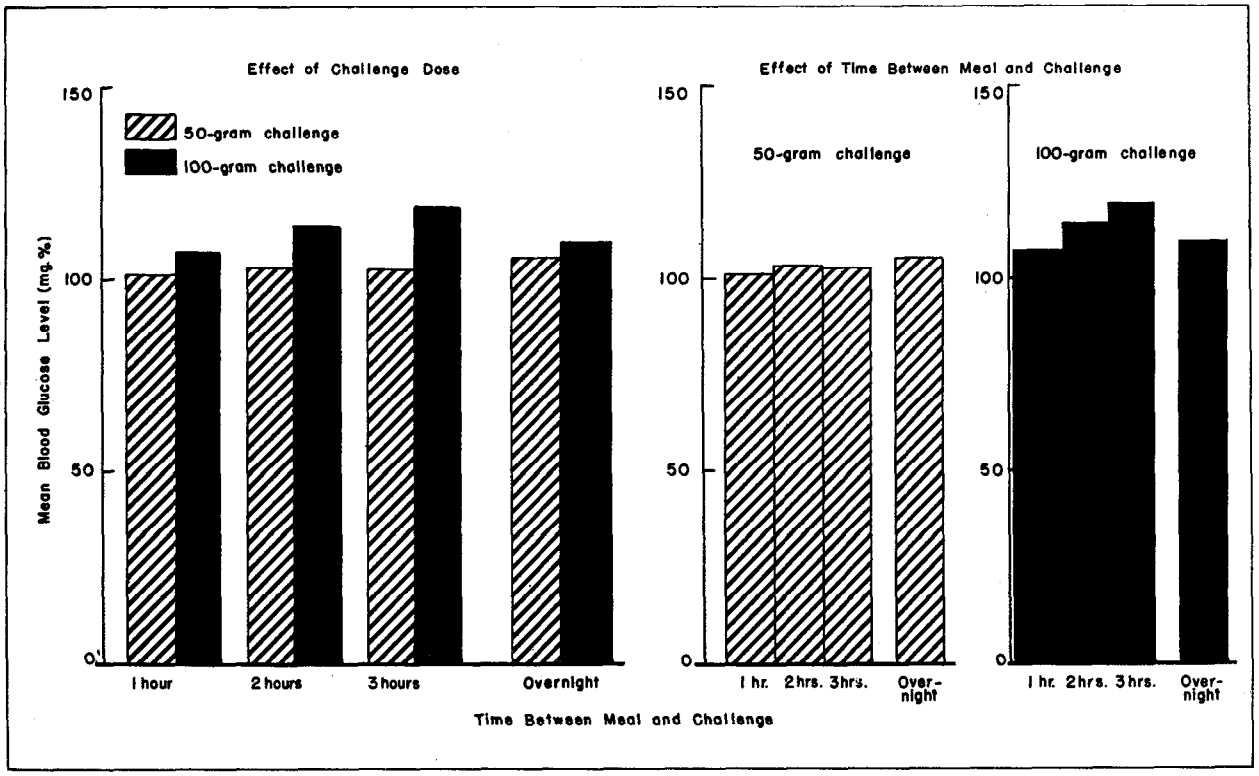

Figure 3. Mean blood glucose level 1 hour after challenge, by various procedures, 24 men, Milan, Phase III.

1, 2, or 3 hours after a meal. However, the difference was not statistically significant for the fasting test. For both the fasting tests and the tests given 1 hour after a meal, only 13 of the 24 persons had a higher response to the 100-gram challenge than the 50 gram. When the challenge was given 2 or 3 hours after a meal, the 1-hour level was, respectively, 11.0 mg.% and 16.5 mg.% higher with a 100-gram challenge than with 50; and was higher in the first instance for 18 of the 24 persons and in the second for 21 of the 24 persons.

These observations refer only to levels 1 hour after challenge. Except where a 100-gram challenge was administered after an overnight fast, this study undertook to measure blood

Table 1. Mean level of blood glucose before challenge and 1 hour after challenge: 24 men, Milan, Phase III

Time from meal to challenge	Mean level (mg.%)				
	Before challenge			1 hour after challenge of	
	Total	100 grams	50 grams	100 grams	50 grams
1 hour	89.5	87.8	91.2	106.8	100.9
2 hours	84.2	83.4	85.0	113.8	102.8
3 hours	77.0	76.8	77.3	118.6	102.1
Overnight	78.4	77.4	79.3	109.4	105.2

glucose levels only before challenge and 1 hour after challenge. It is conceivable therefore that the peak response was as high to the 50-gram challenge as to the 100-gram challenge, but that the peak came at a different time. It will be shown, however, that urine glucose concentrations tended to be higher after a 100-gram challenge than after 50, which would seem to argue for generally higher levels of blood glucose after 100 grams.

The mean blood glucose level 1 hour after a 50-gram challenge appeared to be the same whether the challenge was given to a fasting individual or 1, 2, or 3 hours after a meal.

On the other hand, the mean blood glucose level 1 hour after a 100-gram challenge was affected by the time the challenge was given. If it was given 2 hours after a meal the level was higher than if the challenge was given 1 hour after a meal. The response level was still higher if the challenge was given 3 hours after a meal. While this "trend" was statistically significant in terms of mean levels for the group, it was not compelling for individuals. In fact, it was noted only in six individuals. In 18 cases, however, the response to a 100-gram challenge given 3 hours after a meal was greater than the response to the same challenge given 1 hour after a meal, so that we are justified in considering this effect of time after meal as generally true.

It does not follow, however, that the level after a 100-gram challenge is higher following an overnight fast than it is when challenge is administered 3 hours after a meal. In fact, the data suggest that the level is lower. This difference, however, is not statistically significant and is found in only 14 of the 24 persons tested.

Figure 4 illustrates specific test results for three individuals selected from the low, middle, and high portions of the response scale.

Each of the eight procedures studied was performed twice on each subject in successive weeks. For the eight procedures taken as a group, the differences in variability of level 1 hour after challenge are not statistically significant. However, this conclusion does not allow for the fact

Table 2. Variation of blood glucose levels before challenge and 1 hour after challenge: 24 men, Milan, Phase III

Time from meal to challenge	Variation (mg.%)		
	Before challenge	1 hour after challenge of	
		100 grams	50 grams
	Average of absolute differences		
1 hour	12.0	14.8	20.2
2 hours	12.6	12.3	15.3
3 hours	11.3	23.1	17.3
Overnight	6.4	20.5	14.2
	Standard deviation of response		
1 hour	10.5	13.4	17.3
2 hours	11.2	11.4	14.0
3 hours	10.8	19.9	17.2
Overnight	5.9	18.7	12.1

NOTE: If d is the absolute difference between replicates of a given measure and there are n pairs of replicate measures, the average of the absolute differences is $\frac{\Sigma d}{n}$ and the standard deviation of response is $\sqrt{\frac{\Sigma d^2}{2n}}$

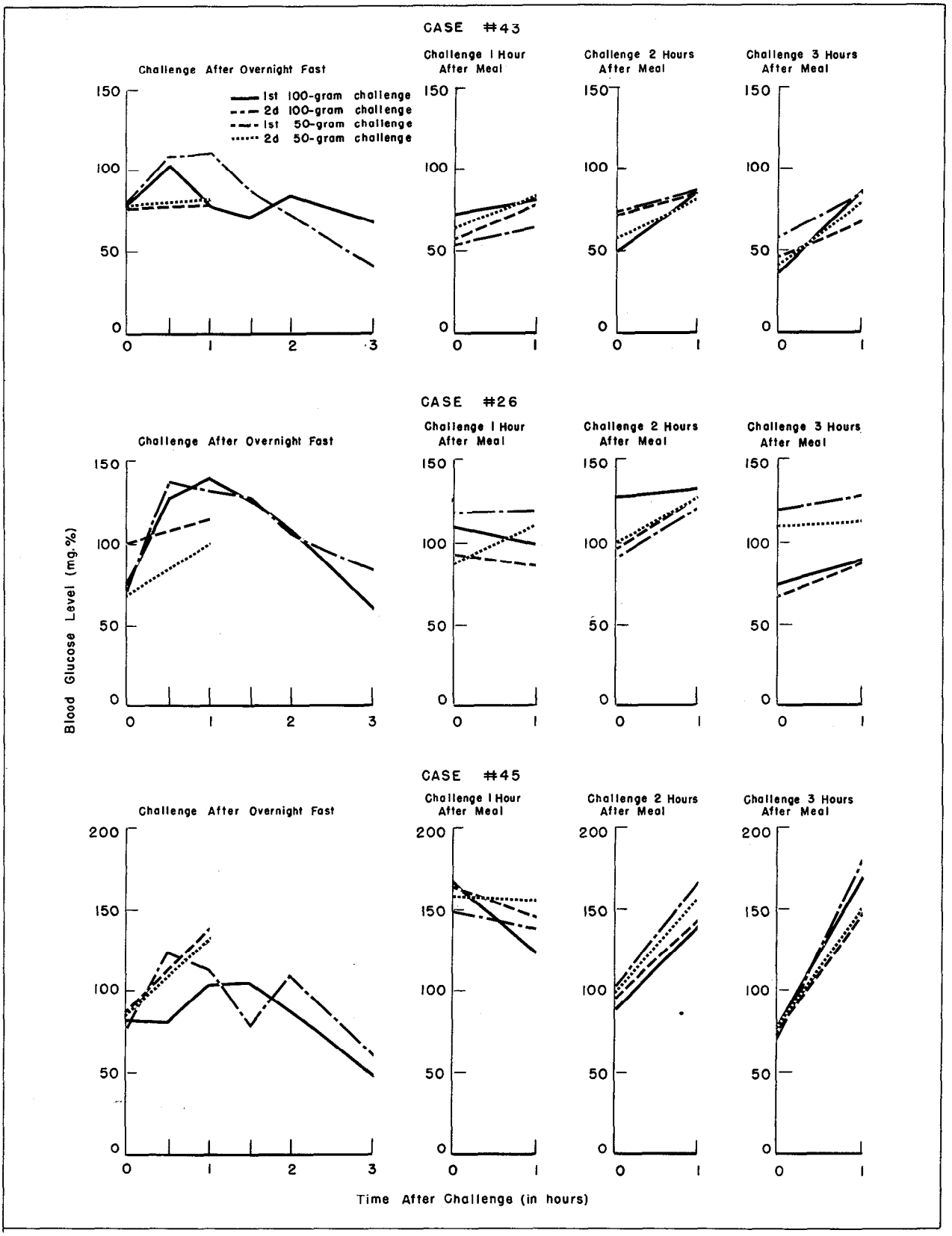

Figure 4. Blood glucose response to specified glucose challenge, Subjects 43, 26, and 45, Milan, Phase III.

that each variance includes a relatively fixed technical variability and that the statistical test is close to the level of significance. In any event, it is well recognized that technical error tends to make it more difficult to demonstrate differences that are actually present. In this case, the most reasonable conclusion from the data is that (appearances to the contrary) the variability in level 1 hour after challenge is not the same for all eight procedures.

Similarly, there is no indication in the data that variability in response is any greater for those persons with high levels of blood glucose than those with low levels. To take a specific example, when a 100-gram challenge is given a fasting individual, the blood glucose level at 1 hour and its variation have a rank correlation of -.05. It cannot even be demonstrated by these data that there is a statistically significant difference in variability of response among different persons. Since both these conclusions are implausible on *a priori* grounds, they may be modified to this statement: In this specific study group, any differences that did exist between persons in their variability of response at 1 hour after challenge could not be demonstrated with the procedures, the laboratory methods, and the extent of replication that was used.

To be conservative then, all statistical tests were performed on the assumption that the variability of level differed from person to person and from procedure to procedure.

THE MEAL

Persons presenting themselves for examination in the usual survey may arrive after a breakfast, lunch, or dinner of variable carbohydrate content, or in a nearly fasting state. It was decided early in planning this study that it would be impossible to evaluate all these factors with the resources available, and that attention would be focused on the effect of giving a challenge at varying times after a meal. However, a description of each participant's previous meal was obtained each time he presented himself during the main study; and the test assignments were arranged so that where the test was given after a meal, half of the study group would always come in after breakfast and the other half after lunch.

The "breakfast" and "lunch" groups were quite similar in glucose response. Their mean blood glucose levels in Phase I, when everyone received a 100-gram challenge after breakfast, were 81.0 and 75.5 mg.% before challenge, and 124.4 and 113.4 mg.% after challenge. Similarly, their morning fasting levels during the main study were nearly identical, 79.8 and 77.5 mg.%; and when a challenge of 100 grams was given after an overnight fast, the blood glucose levels rose to the same level in both groups, 110.3 and 108.5 mg.%. The only discordant note is the difference in response to a 50-gram challenge after an overnight fast, the "breakfast" group

Table 3. Mean level of blood glucose before challenge and 1 hour after challenge, according to meal preceding challenge: 24 men, Milan, Phase III

Time from meal to challenge	Mean level (mg.%)					
	Before challenge		1 hour after challenge of			
			100 grams		50 grams	
	After breakfast	After lunch	After breakfast	After lunch	After breakfast	After lunch
1 hour	86.5	92.5	100.4	113.3	97.2	104.5
2 hours	81.6	86.8	105.4	122.2	96.4	109.2
3 hours	73.4	80.6	110.0	127.1	96.9	107.2

NOTE: 12 men received challenges after breakfast, 12 after lunch.

rising to 101.8 1 hour after challenge and the "lunch" group to 108.6. However, the weight of the evidence favors the conclusion that under the same circumstances the two groups had essentially the same blood glucose levels. Hence where mean levels after a meal differ, it seems reasonable to attribute most of the differences to the content of the meal or to the time of day. Of course, comparisons between tests after breakfast and after lunch will also reflect any diurnal rhythm or differences in recent physical work.

Table 3 gives average levels of response for these two groups under the various test procedures. Blood glucose levels both before challenge and 1 hour after challenge were higher after lunch than after breakfast. When a challenge was given after lunch, the rise was greater than when the same challenge was given after breakfast. The level 1 hour after challenge, when the challenge (either 50 or 100 grams) was given after breakfast, was lower than when the challenge was given to fasting individuals, although the differences were trivial and not statistically significant. Similarly, with a 50-gram challenge given after lunch, the levels 1 hour after challenge were indistinguishable from those obtained from a 50-gram challenge given after an overnight fast. A 100-gram challenge given after lunch, however, yielded 1-hour levels distinctly higher than did the same challenge given after an overnight fast.

All of these differences may, of course, reflect differences between the persons assigned to the two groups (although this is unlikely), but the study was not designed to sort out this kind of factor with great precision. It is worth noting, however, that the carbohydrate intake at breakfast tended to be higher than at lunch, although the kind of carbohydrate eaten at these meals is not the same and may conceivably have different effects on the glucose tolerance test. The range and mean carbohydrate intake during the main study are given for each person in the study group in detailed table IV.

URINE GLUCOSE

A semiquantitative glucose oxidase tape method specific for glucose was used to test urine specimens collected 1½ hours after each of the 16 glucose loading tests performed on each subject. Data are given in detailed table III and summarized in table 4. No negative urine was obtained when the 1-hour blood glucose level was over 160 mg.%, whereas no urine specimen showed even a trace of glucose when the 1-hour blood glucose level was below 60 mg.%.

Nine persons had positive urine with some frequency (at least 7 times out of 16). Their urine glucose findings may be roughly quantified by using the test scale (1, 2, 3, 4), assigning a value of ½ for a glucose trace, and zero for a negative urine. A person's response to the replicates of one procedure may be combined and compared with the parallel statistic for another procedure. If this approach is used to compare all 100-gram tests for these nine persons with all their 50-gram tests, the average score for the 100-gram tests is 0.847 more (the difference having a standard deviation, $Sp/\sqrt{n}$ in the notation of Appendix II, of 0.276). In short, the 100-gram challenge elicited a significantly higher concentration of glucose in the urine of these nine subjects than the 50-gram challenge. This statement also applies to the 24 persons taken as a whole.

THE STANDARD GLUCOSE TOLERANCE TEST

The study yielded a large amount of data relating to the standard glucose tolerance test. In the Phase II pretest, 10 men were given 100-gram challenges twice after an overnight fast, and successive blood specimens were taken. Four of these men were not participants in subsequent tests, but the other 6 and another 18 of the original volunteers did participate in the main study, where the same sort of test was administered in replicate. After Phase III was completed, 22 of these 24 men were also given a single standard glucose tolerance test preceded by a 3-day period of high carbohydrate intake. Thus, there were 28 men with at least one pair of standard glucose tolerance tests and there were 6 men with 5 standard glucose tolerance tests.

These various data are presented in detailed table IV and summarized in table 5 and figure 5. They indicate that while the fasting

blood glucose level of an individual is most stable, each of the levels between ½ and 3 hours after challenge has a standard deviation between 12 and 18 mg.%. This variability, of course, complicates the evaluation of the glucose tolerance test when the test results fall relatively close to whatever critical values are used for diagnosis. To evaluate changes in clinical status on the basis of single standard glucose tolerance tests is especially hazardous in light of the high variability of the individual tests.

It is worth noting that the variability of the ensemble of measurements taken in a standard glucose tolerance test is actually greater than appears from table 5. This may be seen by the following: Add all the blood glucose values for a single standard glucose tolerance test for each of the 28 pairs of 3-hour tests performed during

Table 4. Urine glucose scores: 24 men, Milan, Phase III

Case number	Total urine glucose scores		
	All tests	100 gram tests	50 gram tests
01	4	0	4
04	1	1	0
05	0	0	0
08	1/2	1/2	0
10	0	0	0
11	1/2	1/2	0
20	9	5 1/2	3 1/2
21	38	20	18
23	1/2	1/2	0
26	0	0	0
28	46	27	19
29	1 1/2	0	1 1/2
33	1/2	1/2	0
36	31 1/2	16 1/2	15
42	8	4	4
43	1 1/2	1	1/2
44	20	13	7
45	29 1/2	15 1/2	14
46	9	6 1/2	2 1/2
47	0	0	0
48	7 1/2	5 1/2	2
49	1 1/2	0	1 1/2
50	0	0	0
59	1	1	0

NOTE: Urine determinations are made 90 minutes after challenge. Negative urine is given a score of 0; trace, ½, readings of 1,2,3, or 4 plus are scored 1,2,3, or 4. There were 8 tests with a challenge of 50 grams, 8 with a challenge of 100 grams, 16 tests altogether.

Table 5. Variation of blood glucose levels on standard glucose tolerance tests according to time after challenge: 28 men, Milan, Phases II and III

Time after challenge	Variation (mg.%)	
	Average of absolute differences	Standard deviation of response
0 hour---------	4.4	3.8
1/2 hour-------	16.8	15.2
1 hour---------	20.0	18.0
1 1/2 hours----	14.1	12.5
2 hours--------	11.9	13.5
3 hours--------	16.1	14.7

NOTE: If d is the absolute difference between replicates of a given measure and there are n pairs of replicate measures, the average of the absolute differences is $1/n \Sigma d$ and the standard deviation of response is $\sqrt{\frac{\Sigma d^2}{2n}}$

Tests in Phases II and III were given without a special preparatory diet.

Phases II and III. The standard deviation between replicate sums is 42.0 mg.%. If it is assumed that the variation at one time after challenge is independent of the variation at any other time, the figure computed from table 5 would be 32.8 mg.%; the difference is statistically significant.

As already noted, the glucose tolerance tests done in Phases II and III were undertaken without any special preparatory diet. Such diets were developed to correct any possible caloric or carbohydrate deprivation, either of which tends to reduce tolerance to a standard challenge.[13, 14] As a special check on this factor, a series of standard glucose tolerance tests were performed during Phase IV on all 22 remaining persons who had participated in the main study. Some of the group were given these tests during 1 week of Phase IV; the remainder were given the tests the following week. The test with preparatory diet was done only once on each person. It will be seen from table 6 that the levels for tests given without the 3-day preparatory diets were, if anything, lower than the comparable results with the preparatory diet, although the differences were not statistically significant. Hence, it can be argued that the normal prison fare constituted preparation enough.

A record was made of the carbohydrate content of the last meal for each subject in each of his nonfasting tests. The range and mean carbohydrate content of each subject's meals in Phase III are given in detailed table V. It will be noted that the meals were generally more than adequate in carbohydrates, suggesting that the subjects were actually receiving a diet resembling the customary glucose tolerance preparatory diet. During Phase III, only two subjects gained or lost more than 4 pounds. Selected discordant 1-hour blood sugar values from duplicate tests revealed that about as many are associated with differences in the carbohydrate content of the respective meals in the same direction as with differences in the opposite direction. Thus, variation in the recent carbohydrate intake does not seem to be a suitable explanation for discordant blood glucose values.

In Phase IV, 2 weeks after the standard glucose tolerance test, a cortisone glucose tolerance test was performed and evaluated in accord with the method of Fajans and Conn.[8] This test and the Phase III and Phase IV 3-hour glucose tolerance tests are summarized clinically

Table 6. Mean level of blood glucose on standard glucose tolerance tests with and without 3-day preparatory diet: 24 men, Milan, Phase III and IV

Time after challenge	Mean level (mg.%)	
	Without preparation (Phase III)	With preparation (Phase IV)
0 hour---------	77.6	78.7
1/2 hour-------	111.8	119.0
1 hour---------	108.3	107.3
1 1/2 hours----	97.1	102.2
2 hours--------	91.5	102.1
3 hours--------	68.5	76.5

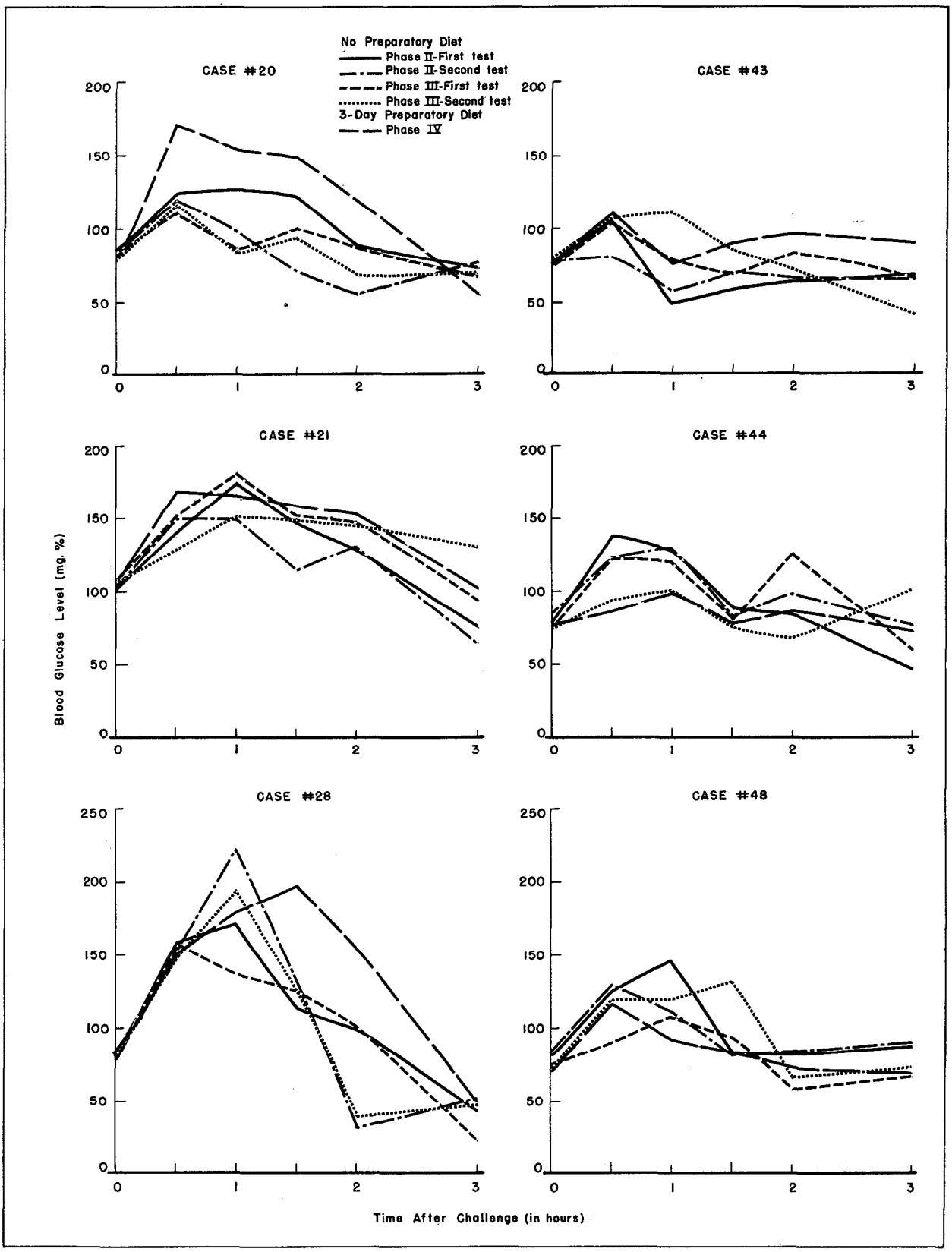

Figure 5. Response to the standard glucose tolerance test, 6 men, Milan, Phases II, III, IV.

for each subject according to established criteria in detailed table VI. Considering as "abnormal" any fasting blood glucose levels above 100 mg.% during Phase III and clinically definite or suspect abnormalities of either of the two procedures in Phase IV, the following four examinees are seen to have manifested definite or suspect evidence of decreased carbohydrate tolerance at least twice: 01, 21, 26, 28.

RANKING INDIVIDUALS

Ultimately, any glucose tolerance test is evaluated by a decision that the blood glucose level is either high or low. Hence, if one glucose tolerance test ranges a set of persons from low to high in the same order as another test, it may be considered as equivalent to that test. If a rank correlation of 1.00 is found between two tests, this means that the individuals are ranked in exactly the same order by both tests. If the rank correlation is 0.00, there is no similarity at all in the order. Where only 24 persons are being evaluated, a rank correlation of 0.34 is indistinguishable from no correlation. For present purposes, negative correlations are equivalent to none.

Table 7 exhibits the mean blood glucose concentration 1 hour after 50-gram and after 100-gram challenge for each person in Phase III and the corresponding ranks. The rank correlation between the average of all 100-gram procedures and the average of all 50-gram procedures is 0.93. Inspection of table 7 and of figure 6 confirms that the 100-gram and 50-gram procedures do, indeed, rank individuals with remarkable consistency.

This does not answer the question of how well a single casual 1-hour test compares with the deliberate test experience. For this purpose the Phase I data may be used. As these were the initial tests performed on each subject and were done at various times after a meal, they are quite comparable to tests performed in epidemiologic surveys. The rank correlation of the average of all 100-gram procedures in Phase III with the single 100-gram test in Phase I was 0.63.

A similar inquiry may be made of the relation of blood glucose levels before and after challenge. There is, of course, a drop in blood

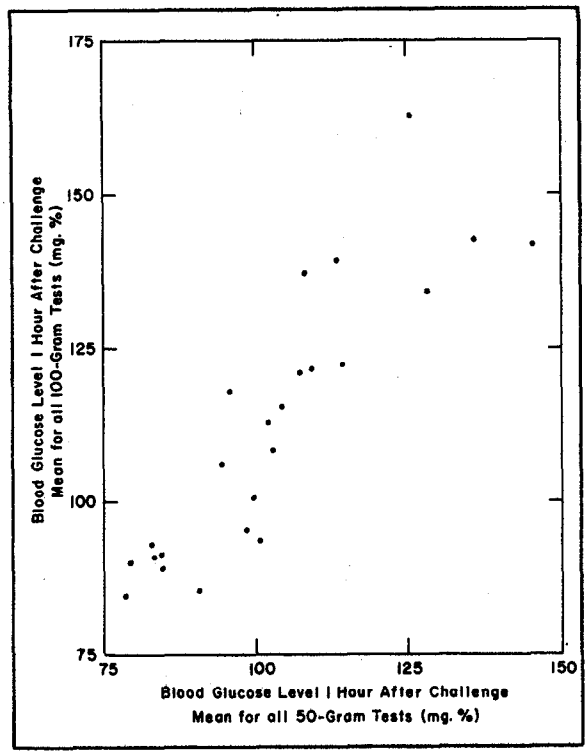

Figure 6. Correlation of mean blood glucose level after the 50-gram challenge with mean blood glucose level after the 100-gram challenge, 24 men, Milan, Phase III.

glucose levels (before challenge) from 1 to 2 to 3 hours after a meal. Nonetheless, it is conceivable that if due allowance is made for shifts in the scale, a casual blood specimen obtained without any deliberate preparation or delay could measure glucose tolerance quite well. Certainly such a test procedure would have distinct advantages for survey work.

In terms of this study, the question can be phrased: how does the blood glucose level before challenge relate to the blood glucose level after challenge, and how is this relationship affected by time since last meal? The rank correlations between levels before challenge and 1 hour after challenge are given in table 8. Needless to say, this study does not allow the complexities of response to carbohydrate challenges in close succession to be evaluated (for that is what a meal followed by a glucose drink amounts to), but it can be said that when the challenge is given within 1 or 2 hours after a meal the correlation of ranks before and after challenge is quite striking.

Table 7. Mean level of blood glucose 1 hour after challenge and ranking of individuals: 24 men, Milan, Phase III

Case number	Mean level (mg.%)			Ranking		
	All tests	100-gram tests	50-gram tests	All tests	100-gram tests	50-gram tests
01	118.3	122.2	114.4	18	18	20
04	87.7	92.3	83.1	6	7	3
05	86.7	88.8	84.6	3	3	6
08	106.8	117.8	95.7	13	15	9
10	87.6	90.8	84.4	5	6	5
11	86.9	90.3	83.5	4	5	4
20	109.4	115.1	103.7	15	14	15
21	139.2	142.4	135.9	22	23	23
23	100.7	106.9	94.5	11	11	8
26	114.9	121.1	108.8	17	17	18
28	122.1	137.0	107.2	19	20	17
29	96.9	94.9	98.8	9	9	10
33	114.0	120.9	107.1	16	16	16
36	144.1	162.7	125.5	24	24	21
42	105.3	108.1	102.4	12	12	14
43	81.6	84.4	78.7	1	1	1
44	107.4	113.0	101.9	14	13	13
45	143.3	141.4	145.1	23	22	24
46	131.0	134.3	127.7	21	19	22
47	87.8	85.2	90.4	7	2	7
48	84.4	89.6	79.3	2	4	2
49	96.8	93.0	100.5	8	8	12
50	99.8	100.3	99.2	10	10	11
59	126.3	139.4	113.2	20	21	19

It is instructive to compare the rank correlations just discussed with the rank correlation of replicated standard glucose tolerance tests. When persons who had fasted overnight were given the 100-gram challenge during Phase III, and this procedure was repeated 1 week later, the rank correlation between their levels 1 hour after challenge was 0.68. This is not surprising, given the high variability of response, but it does raise the question whether this generally accepted standard procedure has much inherent advantage over any of the other seven procedures under investigation.

The urine tests do not lend themselves equally well to rank correlation techniques, since the majority of persons in the study seldom if ever "spilled" glucose into their urine even after an 100-gram challenge and were consequently tied in rank. However, using the scoring system previously described, it is evident by comparison of tables 4 and 7 that the nine persons who tended to "spill" after challenge usually ranked high in blood glucose level after challenge. Figure 7 shows the relation of average blood glucose level 1 hour after challenge to the composite urine glucose score for each of the 24 persons in Phase III.

Table 8. Rank correlation of blood glucose levels before challenge and 1 hour after challenge: 24 men, Milan, Phase III

Time from meal to challenge	Glucose challenge	
	100 grams	50 grams
1 hour	.81	.74
2 hours	.68	.54
3 hours	.40	.21
Overnight	.26	.44

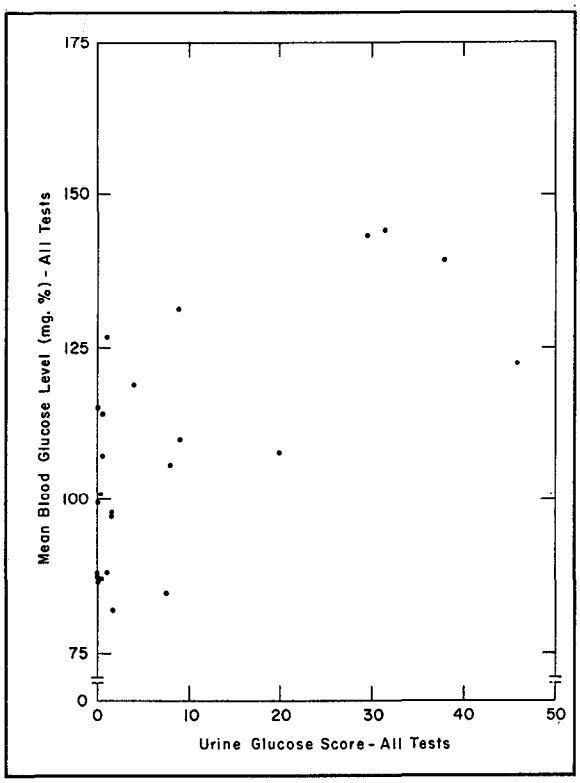

Figure 7. Correlation of mean blood glucose level 1 hour after challenge with urine glucose score 90 minutes after challenge, 24 men, Milan, Phase III.

INCIDENTAL OBSERVATIONS

There were a few occasions in which individual idiosyncrasy seemed to be the explanation for discordant duplicate tests or a peculiarity in the glucose tolerance curve. A discordant pair of values for an individual may arise from labeling errors, failure of examinee to follow instructions, laboratory mistakes or some other defect in techniques, or may only reflect the inherent variability of an individual. That there is no safe way for deciding which factor prevails in a given case is illustrated by data for examinee 28. His four glucose tolerance tests in Phases II and III (shown in figure 5) were highly variable 2 hours after the challenge. On the first test, the level was 100 mg.%; 1 week later it was 30.5 mg.%. This difference of 69.5 mg.% should be compared with the average difference of 14.6 mg.% for all 10 men in Phase II, including examinee 28. However, 3 months later duplicate standard glucose tolerance tests were done on the same man with almost identical results. The first week showed 101.5 mg.% at 2 hours; the second week it was 40.0 mg.%. What on the first pair of tests seems to betoken a technical error, on the second pair of tests may more logically be attributed to examinee idiosyncrasy.

Clearly, in a small series of cases one or two very peculiar individuals can produce a distorted picture of the general population. This does not appear to have happened in this study, for even the idiosyncrasy just mentioned has only a minor effect on the mean values. Therefore, even though clinicians have a natural interest in the unusual case, the kind of study undertaken here can shed little light on cases of this type.

DISCUSSION

One must be most circumspect in generalizing from a study such as this. For one thing, the group was limited in size. For another, this was an unusual group, living under unusual circumstances. The age range was limited; the group included only men. Any number of artifacts might have intruded on the study. Some peculiarity of the prison diet might have affected the results. Usually the subjects spent the hour after glucose loading in comparative idleness during which they often smoked cigarettes. This is quite different from the examination routine of either the Tecumseh Study or the Health Examination Survey, in both of which participants are occupied during the period between challenge and venipuncture and have little opportunity for smoking.

There was an epidemic of Type B influenza during the eighth and ninth weeks of Phase III. Each subject was routinely asked at each session whether he had a cold or fever or other infection, and it was only during these weeks that any excess number of respiratory infections was noted in the study group. However, the subjects reporting these symptoms revealed no consistent alteration in response to glucose load in comparison with asymptomatic periods.

One disturbing feature of the study group is the low mean blood glucose level after challenge. The response to a 100-gram challenge is distinctly less than noted in the Tecumseh Study, while the response to a 50-gram challenge is less

than that found in the Health Examination Survey. On the other hand, their response levels were comparable to those found by Wilkerson and his associates[14] in another prisoner group. There does not seem to be an obvious explanation for these findings.

These various qualifications are not entered to deprecate any findings of this study. In the last analysis, no study can stand by itself. It must be integrated with the findings of other related studies and must be repeated by other investigators on other study groups before its meaning becomes clear and certain.

While there have no doubt been numerous informal observations made of the factors investigated in this study, there are relatively few solid data in the literature. Maclean[15], an early worker with the glucose tolerance test, observed that ". . . after a certain dose is reached, about 25 grams, further increase in the amount of sugar does not increase the actual height of the resulting hyperglycaemia." Of course, laboratory techniques then in use measured something more than blood glucose, so that his findings are not necessarily in contradiction to this study. In any event one clear finding in this study is that a 100-gram challenge yields a somewhat higher blood glucose level than a 50-gram challenge.

Irving and Wang,[16] in a study which essentially yielded replicate standard glucose tolerance tests on a series of 12 persons, found, as in this study, large variability in the results. The variability in level of their subjects, while somewhat greater than that for subjects in this study at fasting and at ½, 1, 1½, and 2 hours after challenge, appears to be of about the same magnitude. What differences do exist may be accounted for by two facts: (1) the measurement of blood glucose concentration in their study was done on capillary blood and could be expected to have a greater measurement variability than determinations made in this study; and (2) the prior preparation was deliberately varied from one test to the other. A study of replicate standard glucose tolerance tests was made by Freeman, Looney, and Hoskins[17] on 35 men, 30 of whom were schizophrenic. Blood glucose was determined by the Folin-Wu method. The average difference between replicate specimens taken fasting and ½, 1, 2, and 3 hours after challenge was 9.0, 25.8, 29.9, 20.3, and 15.2 mg.%. These are all greater than the comparable figures for our study group. If it is assumed that the fasting glucose level is highly stable, the greater variability reported in fasting tests in their study suggests a greater technical variability in the measuring technique than obtained in this study.

Unger[18] studied the variability of standard glucose tolerance tests using a group of food handlers with a casual postprandial blood glucose level on screening of less than 130 mg.%. "Within-person" standard deviations may be computed from his published data. For 7 men under age 40 and for 10 men over 40 the standard deviations of the 1-hour blood glucose level were 23.9 and 29.7 mg.%, respectively. The corresponding figures for 15 women under 40 and 17 women over 40 were 32.8 and 25.9 mg.%. The figure for the inmate volunteers in this study was 18.0 mg.%. Again, part of the difference may be technical, since the standard deviation of fasting levels was also higher than in this group. For men over 40 it was 7.6 mg.% as compared with 3.8 mg.% for this study group. At 2 hours, however, the standard deviation for men over 40 in Unger's group was 6.7 mg.% as contrasted with 11.7 mg.% for this group.

Summing up the various comparisons, it appears that the variability of response to challenge found in this study, high though it was, probably represents a conservative estimate of this factor.

One interesting finding in this study is that the response to a 50-gram challenge appears to be quite insensitive to the interval since prior meal. Whether the 50-gram challenge is given fasting or 1, 2, or 3 hours after a meal the blood glucose level 1 hour after challenge appears to be the same. Data from the Health Examination Survey suggest that the effect of time from last meal to a 50-gram challenge is not as trivial as appears from this study, and there are some anomalies in the results of the Milan Study itself which suggest special caution be used in interpreting the findings with respect to the 50-gram challenge. Still, the effect of time after meal seems definitely greater with a 100-gram challenge than with 50.

Finally, it must be said quite explicitly that this study cannot be used to decide whether any specific tolerance test is best for determining the presence or absence of diabetes. What the

study does strongly suggest is that any of the procedures under investigation will tend to rank persons with respect to glucose tolerance in about the same order from low to high, that a casual glucose tolerance procedure yields results quite similar to a standardized procedure, but that any procedure will yield variant results when repeated on the same individual.

SUMMARY AND CONCLUSIONS

Using 24 male prisoner volunteers 40-52 years of age as subjects, 1-hour oral glucose tolerance tests performed under eight different arrangements were compared. Challenges were given with both 50 and 100 grams of glucose, and were given after an overnight fast and 1, 2, and 3 hours after breakfast or lunch. Each procedure was performed twice. The 16 tests for each subject were performed at weekly intervals. Fasting 100-gram tests were extended to 3 hours. Subsequently, 22 of the subjects were given three clinical tests after added dietary carbohydrate: the standard glucose tolerance test, the cortisone glucose tolerance test, and the prednisone glycosuria test. The following findings were noted:

A challenge of 100 grams of glucose yielded slightly but consistently higher mean blood glucose levels 1 hour after challenge, and significantly higher concentrations of urine glucose, than did a 50-gram challenge.

Despite this fact, individuals with high levels 1 hour after a 100-gram challenge also had relatively high levels after a 50-gram challenge and mean response to the two loads appeared to rank individuals in almost the same order. The four subjects classified clinically as exhibiting some evidence of deficient carbohydrate tolerance were ranked high by both the 50-gram and 100-gram tests.

In contrast with the more uniform 1-hour levels of the group given 100 grams of glucose at various intervals after breakfast, response levels of the apparently similar group tested after lunch with 100-gram challenges increased with time after meal. On the other hand, the 50-gram test revealed no significant correlation of response level with interval after meal in either group. These findings should be treated with some reserve.

An individual's blood glucose level after overnight fast was highly stable but his level under other circumstances was variable. In particular, the variability of response to challenge after an overnight fast was of the same magnitude as variability of response when the challenge was administered after a meal.

Administration of a glucose challenge whenever a person comes in for examination, no matter when or what he last ate, appears to be an entirely reasonable method of testing for carbohydrate tolerance.

REFERENCES

[1]J. H. Moyer and C. R. Womack, "Glucose Tolerance: A Comparison of Four Types of Diagnostic Tests," *American Journal of Medical Sciences,* Vol. 219, No. 2, 1950, p. 161.

[2]J. W. Conn and S. S. Fajans, "The Prediabetic State," *American Journal of Medicine,* Vol. 31, 1961, p. 839.

[3]U. S. National Health Survey, "Plan and Initial Program of the Health Examination Survey," *Health Statistics,* Series A, No. 4. Washington, U. S. Government Printing Office, 1962.

[4]J. A. Napier, "Field Methods and Response Rates in the Tecumseh Community Health Study," *American Journal of Public Health,* Vol. 52, 1962, p. 208.

[5]J. M. Leichsenring and E. D. Wilson, "Food Composition Table for Short Method of Dietary Analysis" (2d revision), *Journal of the American Dietetic Association,* Vol. 27, 1951, p. 386.

[6]A. de P. Bowes and C. F. Church, *Food Values of Portions Commonly Used,* 8th edition. Philadelphia, Lippincott, 1956.

[7]B. K. Watt and A. L. Merrill, *Composition of Foods--Raw, Processed, Prepared,* U. S. Department of Agriculture Handbook No. 8. Washington, U. S. Government Printing Office, 1950.

[8]S. S. Fajans and J. W. Conn, "The Early Recognition of Diabetes Mellitus," *Annals of the New York Academy of Sciences,* Vol. 82, 1959, p. 208.

[9]F. G. Joplin, R. Fraser, and K. J. Keeley, "Prednisone-Glycosuria Test for Prediabetes," *Lancet,* Vol. II for 1961, p. 67, 1961.

[10]M. Somogyi, "Determination of Blood Sugar," *Journal of Biological Chemistry,* Vol. 160, 1945, p. 69.

[11]J. B. O'Sullivan, N. Kantor, and H. L. C. Wilkerson, "Comparative Value of Tests for Urinary Glucose," *Diabetes,* Vol. 11, 1962, p. 53.

[12]E. R. Froesch and A. E. Renold, "Specific Enzymatic Determination of Glucose in Blood and Urine Using Glucose Oxidase," *Diabetes,* Vol. 5, 1956, p. 1.

[13]J. W. Conn, "Interpretation of Glucose Tolerance Test," *American Journal of Medical Sciences,* Vol. 199, 1940, p. 555.

[14]H. L. C. Wilkerson, H. Hyman, M. Kaufman, A. C. McCuiston, and J. O'S. Francis, "Diagnostic Evaluation of Oral Glucose Tolerance Tests in Non-Diabetic Subjects After Various Levels of Carbohydrate Intake," *New England Journal of Medicine,* Vol. 262, No. 21, 1960, p. 1047.

[15]Hugh Maclean, *Modern Methods in the Diagnosis of Treatment of Glycosuria and Diabetes,* 5th edition. London, Constable and Company, Ltd., 1932.

[16]E.M. Irving and I. Wang, "The Effect of the Previous Diet on Glucose Tolerance Tests," *Glascow Medical Journal,* Vol. 35, No. 11, 1954, p. 275.

[17]H. Freeman, J. M. Looney, and R. G. Hoskins, "Spontaneous Variability of Oral Glucose Tolerance," *Journal of Clinical Endocrinology and Metabolism,* Vol. 2, 1942, p. 431.

[18]R. H. Unger, "The Standard Two-Hour Oral Glucose Tolerance Test in the Diagnosis of Diabetes Mellitus in Subjects Without Fasting Hyperglycemia," *Annals of Internal Medicine,* Vol. 47, 1957, p. 1138.

DETAILED TABLES

Table I. Blood glucose and urine glucose findings: 45 men, Milan, Phase I

Case number	First week			Case number	Second week		
	Blood glucose levels		Urine glucose 1½ hours after challenge		Blood glucose levels		Urine glucose 1½ hours after challenge
	Before challenge	1 hour after challenge			Before challenge	1 hour after challenge	
01[a]	74.0	116.0	Negative	31	74.0	80.0	Negative
02	107.5	84.5	Negative	32	103.0	100.5	Negative
03	76.5	64.0	Trace	33[a]	87.0	120.0	Negative
04[a]	67.5	97.0	Negative	34	77.0	82.0	Negative
05[a]	95.0	78.0	Negative	35[d]	79.5	138.0	1+
06	85.5	83.0	2+	36[a]	65.0	121.5	2+
07	134.0	100.0	2+	37	82.5	83.0	Negative
08[a]	86.5	96.5	Negative	38	69.5	102.0	Trace
09[c]	81.5	120.0	4+	39	70.0	87.0	Negative
10[a]	87.5	90.0	Negative	40	69.0	82.0	Negative
11[a]	78.0	88.5	Negative	41	92.0	95.5	Negative
19[b]	66.0	114.0	Negative	42[a]	69.5	102.0	Negative
20[a]	87.5	226.0	2+	43[a]	87.0	75.0	Negative
21[a]	68.0	158.0	2+	44[a]	66.0	155.0	3+
22	91.5	87.5	Negative	45[a]	80.0	144.5	2+
23[a]	69.0	113.0	Negative	46[a]	73.5	132.0	Negative
24	54.5	79.0	Negative	47[a]	82.5	99.5	Negative
25	96.0	86.0	Negative	48[a]	100.5	127.0	Negative
26[a]	70.0	122.0	Negative	49[a]	69.0	133.0	Negative
27	82.0	106.0	2+	50[a]	88.5	118.0	Negative
28[a]	77.0	144.0	3+	58	69.0	83.5	Negative
29[a]	77.0	75.0	Negative	59[a]	72.0	122.0	Negative
30[c]	78.0	56.0	Negative				

[a]Selected for Phase III.

[b]Selected for Phase III but discharged and replaced by case number 29.

[c]Veins unsuitable for venipuncture.

[d]Selected for Phase III but discharged and replaced by case number 08.

Table II. Assignment of subjects to experimental procedures: 24 men, Milan, Phase III

Case number	Week							
	1, 2	3, 4	5, 6	7, 8	9, 10	11, 12	13, 14	15, 16
Morning group								
28, 10, 50	D	H	F	G	B	A	C	E
21, 48, 11	H	D	A	C	E	F	G	B
49, 33, 04	A	F	H	E	C	D	B	G
20, 01, 43	F	A	D	B	G	H	E	C
Afternoon group								
46, 59, 42	B	E	G	F	D	C	A	H
45, 26, 05	E	B	C	A	H	G	F	D
44, 36, 47	C	G	E	H	A	B	D	F
08, 29, 23	G	C	B	D	F	E	H	A

Key to Procedures

A = 100 grams challenge after overnight fast
B = 50 grams challenge after overnight fast
C = 100 grams challenge 1 hour after meal
D = 50 grams challenge 1 hour after meal
E = 100 grams challenge 2 hours after meal
F = 50 grams challenge 2 hours after meal
G = 100 grams challenge 3 hours after meal
H = 50 grams challenge 3 hours after meal

Lapses:

Case number 01, 04, 10, 11, 20, 28, 33, 42, 43, 48, 49, 50—no lapses

Case number 05,21,26,44,45, 47 were given challenges of 100 grams in week 13, when they should have been given challenges of 50 grams. These results were discarded and the correct procedures were done on week 17.

Case number 46 was ill week 15. This procedure was completed week 17.

Case number 59. The values obtained during week 15 were considered highly improbable for this person and discarded. The procedure was completed on week 18.

Case number 36. The values obtained on week 15 were considered highly improbable for this person and discarded. On week 13 this person was given 100 grams of glucose instead of 50. These losses could not be made up later.

Case number 23. The value before challenge obtained on week 16 was considered highly improbable for this person and discarded. On week 13 this person was given 100 grams of glucose instead of 50. This procedure was completed on week 17.

Case number 29. Replaced case number 19, the original assignee, after week 1. Week 1 procedure was completed (by accident) on week 13. Week 13 procedure was completed week 17.

Case number 08. Replaced case number 35, the original assignee, after week 5. It proved impossible to make up all the lost ground. Week 1 procedure was completed week 13. Week 3 procedure was completed week 17. Week 13 procedure was completed week 15. Week 15 procedure was completed week 19. Duplicates of procedures B and C (assigned to weeks 5 and 4,respectively) were never completed.

NOTE: Letters shown in body of table refer to procedure.

Table III. Blood glucose and urine glucose findings according to procedure used: 24 men, Milan, Phase III

Case number	A		B		C		D		E		F		G		H	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
	Blood glucose level (mg. %) before challenge															
01---------	77.5	70.5	99.0	103.0	102.0	113.0	89.0	112.5	80.0	81.5	73.5	68.0	69.0	74.0	79.0	75.0
04---------	69.5	66.5	77.0	67.0	62.0	60.5	79.0	58.0	60.0	82.0	75.0	60.5	50.5	63.0	86.0	63.5
05---------	74.5	73.5	88.5	82.5	55.0	56.5	69.0	57.0	58.0	75.0	78.0	74.5	91.0	80.5	76.5	67.5
08---------	79.0	87.5	74.5	---	65.0	---	93.0	90.0	89.0	88.0	95.0	97.5	99.5	83.0	75.5	71.0
10---------	74.0	70.0	75.0	65.5	64.5	67.0	78.0	48.5	65.0	63.0	86.0	67.0	76.5	66.0	78.0	77.5
11---------	71.0	80.0	63.0	76.5	70.5	64.5	72.5	72.5	91.0	88.5	82.0	74.5	97.0	78.0	89.0	90.5
20---------	83.5	81.5	78.5	66.0	100.5	103.5	72.0	101.0	95.0	73.0	82.0	111.0	65.5	83.0	76.0	63.5
21---------	109.0	102.5	104.0	114.5	127.5	104.0	156.5	150.5	135.0	126.5	111.5	141.0	108.0	111.5	104.0	131.0
23---------	76.0	---	80.5	72.5	100.5	96.0	101.0	78.0	82.0	71.5	97.0	97.0	91.5	62.5	89.0	92.0
26---------	75.0	72.0	100.5	68.0	110.0	120.0	92.0	87.5	129.5	91.0	97.5	101.5	74.5	120.5	67.0	110.0
28---------	78.5	79.0	71.0	80.0	132.0	135.0	108.5	104.0	72.0	110.0	85.0	72.5	75.5	67.0	56.5	55.0
29---------	65.5	68.5	75.0	75.0	84.0	68.0	90.5	71.0	71.5	59.0	64.0	70.0	67.0	61.0	63.5	71.0
33---------	76.5	71.0	70.0	78.0	96.5	99.0	93.0	101.0	64.0	90.5	111.0	87.0	69.0	84.5	72.0	81.0
36---------	66.0	68.5	66.0	69.0	102.0	90.0	111.5	---	79.0	83.5	77.0	---	72.5	84.0	97.5	71.5
42---------	83.0	86.0	91.5	85.5	76.0	66.5	79.0	72.0	98.0	87.5	86.5	92.5	89.5	89.5	84.0	109.0
43---------	78.0	81.5	77.0	79.5	72.0	55.0	56.0	62.5	49.5	75.0	72.0	57.5	35.5	57.5	46.0	41.0
44---------	74.5	74.0	74.0	79.0	63.0	65.0	95.5	91.0	78.0	93.5	77.0	76.0	76.5	86.5	78.0	83.0
45---------	81.5	79.5	86.0	85.0	166.5	148.0	163.0	156.5	89.5	104.5	95.0	100.0	79.0	71.5	72.0	78.5
46---------	73.0	83.5	71.5	84.0	97.5	78.5	113.0	129.0	94.5	99.5	82.0	97.0	84.0	70.0	67.0	73.5
47---------	75.0	68.0	70.5	77.5	70.5	80.0	76.0	87.0	88.5	80.0	102.0	79.0	74.5	78.5	81.0	82.0
48---------	75.0	74.0	69.0	72.0	55.5	66.5	68.0	89.0	79.0	63.0	82.5	70.0	57.5	58.0	69.5	76.0
49---------	74.0	78.5	73.5	70.5	70.5	74.0	55.0	79.5	65.5	69.0	58.5	64.5	63.5	86.0	62.0	60.5
50---------	86.5	99.0	104.0	92.0	98.5	87.5	65.5	99.5	69.5	82.0	100.5	93.0	68.0	71.0	81.0	76.5
59---------	77.5	72.0	70.0	80.5	93.5	116.0	87.0	104.5	88.0	93.0	101.0	82.0	82.0	83.5	74.0	85.0

Table III. Blood glucose and urine glucose findings according to procedure used: 24 men, Milan, Phase III—Con.

Case number	A		B		C		D		E		F		G		H	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
	Blood glucose level (mg. %) 1 hour after challenge															
01---------	147.0	126.5	136.0	100.0	108.0	111.5	98.5	148.5	134.5	104.0	89.0	102.0	149.5	96.5	131.5	110.0
04---------	93.0	110.0	66.5	87.5	83.5	91.0	94.0	76.0	94.0	82.0	84.0	82.5	90.0	94.5	101.5	73.0
05---------	72.0	86.0	93.5	72.5	78.0	86.5	77.0	82.5	69.0	63.5	104.5	74.5	134.5	121.0	86.5	86.0
08---------	115.5	121.5	109.0	---	140.0	---	98.0	76.0	94.0	115.0	92.5	96.5	102.5	114.0	105.0	79.5
10---------	80.0	81.0	85.5	80.0	99.0	77.5	83.5	94.5	77.5	91.5	76.5	94.5	118.0	101.5	82.5	78.5
11---------	102.5	101.0	66.0	92.0	83.5	89.0	98.5	89.0	85.0	82.0	97.0	79.0	101.0	78.5	78.0	68.5
20---------	86.5	84.0	111.0	101.0	108.5	123.5	83.5	107.5	132.0	147.5	83.0	89.0	91.0	148.0	127.5	127.0
21---------	181.0	151.0	140.5	143.0	133.0	95.0	149.5	136.5	145.0	150.0	124.5	148.0	156.5	127.5	131.5	114.0
23---------	141.5	109.5	107.5	114.5	107.0	108.5	86.5	69.0	87.5	87.0	78.5	111.0	91.5	122.5	93.5	95.5
26---------	140.5	134.0	114.5	100.0	100.0	121.0	86.0	112.0	133.0	121.0	129.0	129.0	89.0	130.0	88.0	112.0
28---------	136.5	195.0	143.5	133.0	117.0	137.5	107.5	83.5	108.5	137.5	100.0	129.5	113.0	151.0	86.0	74.5
29---------	75.0	83.0	109.5	114.0	107.0	112.0	102.5	64.0	96.5	104.0	98.0	100.5	84.5	97.5	58.5	143.5
33---------	128.0	86.5	94.0	95.0	126.0	143.5	100.0	119.0	107.0	142.0	99.5	115.5	124.5	110.0	125.0	108.5
36---------	137.5	112.0	81.5	99.0	137.0	124.5	110.5	---	197.5	195.5	150.0	---	214.0	183.5	154.0	148.5
42---------	90.0	105.5	102.5	96.0	92.5	85.5	96.5	99.5	117.0	125.0	125.5	106.5	112.5	136.5	84.5	108.5
43---------	78.0	110.0	78.0	81.0	81.0	64.5	79.0	82.0	84.0	86.0	84.0	81.0	86.5	85.5	67.0	77.5
44---------	120.5	101.0	108.0	120.5	83.0	133.0	130.5	104.0	101.0	109.0	93.0	92.0	147.0	109.5	69.0	98.0
45---------	103.0	113.0	137.0	130.0	124.0	138.5	146.5	156.0	140.0	165.5	142.5	156.0	169.5	178.0	146.0	147.0
46---------	126.5	126.5	125.5	159.5	104.0	127.0	128.0	118.0	159.5	165.0	109.0	137.0	131.0	135.0	131.0	113.5
47---------	88.5	51.5	84.5	94.5	80.5	82.0	112.0	116.0	99.0	95.0	70.0	70.5	118.5	66.5	94.5	81.0
48---------	108.5	119.5	91.0	71.5	76.0	78.0	94.5	79.0	88.5	62.0	60.0	75.0	104.0	80.0	78.5	85.0
49---------	41.5	103.5	112.0	127.0	95.5	83.5	65.5	111.5	108.0	110.0	63.5	113.5	106.5	95.5	103.0	108.0
50---------	85.0	111.0	118.0	90.0	117.0	87.0	65.5	87.0	75.0	95.0	113.0	130.0	113.0	117.0	110.0	80.0
59---------	132.5	118.0	118.0	105.0	150.0	157.0	90.0	137.5	148.0	146.5	108.0	97.5	137.5	125.5	111.5	138.0

Table III. Blood glucose and urine glucose findings according to procedure used: 24 men, Milan, Phase III—Con.

Case number	A		B		C		D		E		F		G		H	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
	Urine glucose 1½ hours after challenge															
01---------	N	N	N	N	N	N	N	2+	N	N	N	2+	N	N	N	N
04---------	N	N	N	N	N	N	N	N	1+	N	N	N	N	N	N	N
05---------	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
08---------	Tr	N	N	---	N	---	N	N	N	N	N	N	N	N	N	N
10---------	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11---------	N	N	N	N	N	Tr	N	N	N	N	N	N	N	N	N	N
20---------	N	Tr	N	N	N	N	N	N	Tr	2+	N	2+	Tr	1+	Tr	1+
21---------	2+	2+	3+	1+	3+	1+	3+	2+	3+	3+	4+	2+	2+	2+	N	2+
23---------	N	N	N	N	N	N	N	N	Tr	N	N	N	N	N	N	N
26---------	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
28---------	4+	4+	3+	3+	4+	2+	1+	3+	4+	2+	1+	3+	3+	3+	2+	2+
29---------	N	N	N	N	N	N	Tr	N	N	N	N	1+	N	N	N	N
33---------	N	N	N	N	N	Tr	N	N	N	N	N	N	N	N	N	N
36---------	3+	Tr	N	N	N	3+	2+	---	3+	2+	3+	---	2+	3+	2+	3+
42---------	N	Tr	N	2+	Tr	N	Tr	N	1+	2+	1+	Tr	N	N	N	N
43---------	N	1+	Tr	N	N	N	N	N	N	N	N	N	N	N	N	N
44---------	1+	1+	1+	N	N	3+	1+	1+	2+	1+	2+	1+	3+	2+	1+	N
45---------	N	Tr	1+	1+	2+	2+	2+	2+	3+	3+	2+	1+	2+	3+	2+	3+
46---------	N	1+	Tr	N	Tr	Tr	1+	N	Tr	2+	N	1+	1+	1+	N	N
47---------	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
48---------	Tr	N	N	N	2+	Tr	N	1+	N	N	1+	N	2+	Tr	N	N
49---------	N	N	N	N	N	N	N	1+	N	N	N	Tr	N	N	N	N
50---------	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
59---------	N	N	N	N	N	N	N	N	N	N	N	N	N	1+	N	N

NOTES: Letters refer to procedure used (see key shown on table II). Numbers 1 or 2 rerer to the time (first or second) that the procedure was administered.

N - negative; tr - trace

Table IV. Standard glucose tolerance tests: 28 men, Milan, Phases II, III, and IV

Case number and test series	Blood glucose levels (mg. %) by time after challenge						Urine glucose	
	0 hour	½ hour	1 hour	1½ hours	2 hours	3 hours	1½ hours after challenge	3 hours after challenge
Case 01								
Phase III, 1	77.5	132.0	147.0	107.5	113.5	66.0	Negative	Negative
III, 2	70.5	144.0	126.5	111.0	103.5	61.0	Negative	---
IV	75.5	118.5	126.0	129.0	91.0	104.0	Negative	Negative
Case 03								
Phase II, 1	79.0	89.5	74.5	91.0	77.0	76.5	1+	---
II, 2	75.0	75.5	68.5	66.0	77.0	75.5	Negative	Negative
Case 04								
Phase III, 1	69.5	106.5	93.0	75.0	93.0	70.0	Negative	---
III, 2	66.5	102.5	110.0	66.0	92.0	75.0	Negative	Negative
IV	73.5	107.5	73.5	56.5	65.5	63.5	1+	Trace
Case 05								
Phase III, 1	74.5	81.0	72.0	80.5	65.0	76.0	Negative	Negative
III, 2	73.5	84.5	86.0	61.5	81.0	73.5	Negative	Negative
IV	81.0	86.0	78.5	72.0	75.0	77.0	Negative	Negative
Case 08								
Phase III, 1	87.5	114.0	121.5	99.5	127.0	102.5	Trace	Trace
III, 2	79.0	107.0	115.5	119.0	118.5	98.0	Negative	Negative
Case 09								
Phase II, 1	78.5	163.0	140.0	115.0	103.0	74.0	2+	---
II, 2	80.0	123.5	149.0	145.0	114.5	96.5	2+	1+
Case 10								
Phase III, 1	74.0	81.0	80.0	74.0	81.0	48.0	Negative	Negative
III, 2	70.0	79.0	81.0	88.5	85.5	54.0	Negative	Negative
IV	73.0	98.0	77.5	76.0	98.0	82.0	Negative	Negative
Case 11								
Phase III, 1	71.0	89.0	102.5	94.0	84.0	89.0	Negative	Negative
III, 2	80.0	94.0	101.0	---	95.0	79.0	---	Negative
IV	93.5	113.5	104.0	90.0	86.0	83.0	Negative	Negative
Case 20								
Phase II, 1	86.0	124.5	125.5	122.5	88.5	74.0	Trace	---
II, 2	84.0	121.0	99.0	72.0	55.0	77.0	Trace	0
III, 1	83.5	112.5	86.5	100.5	88.0	68.5	Negative	---
III, 2	81.5	117.5	84.0	95.0	68.5	69.5	Trace	---
IV	79.5	171.5	153.0	150.0	119.5	55.5	3+	1+
Case 21								
Phase II, 1	100.5	140.0	175.0	147.5	128.0	75.0	3+	---
II, 2	102.0	150.0	150.0	114.5	129.5	64.5	4+	2+
III, 1	109.0	152.5	181.0	151.5	148.0	94.0	2+	1+
III, 2	102.5	---	151.0	150.0	146.0	130.5	2+	2+
IV	108.5	168.5	165.5	159.0	153.0	102.5	3+	4+
Case 23								
Phase III, 1	76.0	127.5	141.5	118.5	102.0	86.0	Negative	Negative
III, 2	---	87.0	109.5	96.0	120.5	88.5	Negative	Negative
IV	90.5	90.0	93.5	103.0	113.5	69.0	Negative	Negative
Case 26								
Phase III, 1	75.0	129.0	140.5	128.0	109.0	59.5	Negative	Negative
III, 2	72.0	139.0	134.0	129.0	106.0	84.5	Negative	Negative
IV	82.5	161.5	169.0	148.5	118.5	59.5	Trace	Trace
Case 28								
Phase II, 1	77.0	158.0	171.5	113.0	100.0	44.5	3+	---
II, 2	83.5	151.0	223.0	132.0	30.5	50.5	---	0
III, 1	78.5	157.5	136.5	126.5	101.5	21.5	4+	---
III, 2	79.0	148.5	195.0	126.5	40.0	46.0	4+	3+
IV	79.5	149.5	180.0	198.5	154.0	48.0	4+	3+
Case 29								
Phase III, 1	65.5	89.5	75.0	65.5	69.0	77.5	Negative	Negative
III, 2	68.5	99.0	83.0	103.0	73.0	68.0	Negative	Negative
IV	78.0	65.0	74.5	45.0	74.0	67.5	Negative	Negative

Table IV. Standard glucose tolerance tests: 28 men, Milan, Phases II, III, and IV—Con.

Case number and test series	Blood glucose levels (mg. %) by time after challenge						Urine glucose	
	0 hour	½ hour	1 hour	1½ hours	2 hours	3 hours	1½ hours after challenge	3 hours after challenge
Case 30								
Phase II, 1	77.0	77.5	83.5	85.0	78.5	82.0	Negative	---
II, 2	78.5	118.0	99.5	100.5	82.5	54.0	Negative	Negative
Case 31								
Phase II, 1	74.0	96.0	87.0	80.5	86.0	88.0	Trace	---
II, 2	81.5	63.5	75.0	98.0	79.0	63.0	Negative	Negative
Case 33								
Phase III, 1	76.5	125.0	128.0	100.5	100.0	92.0	Negative	---
III, 2	71.0	115.0	86.5	116.0	99.0	51.5	Negative	Negative
IV	74.5	149.5	115.0	90.5	94.5	71.5	Negative	Negative
Case 36								
Phase III, 1	66.0	161.5	137.5	111.0	77.0	37.5	3+	1+
III, 2	68.5	128.5	108.0	116.5	78.0	45.0	Trace	Negative
Case 42								
Phase III, 1	83.0	106.5	90.0	82.0	93.5	81.0	Negative	Trace
III, 2	86.0	103.0	105.5	88.0	103.0	108.0	Trace	Negative
IV	83.5	102.5	116.5	110.5	121.0	106.5	Negative	Negative
Case 43								
Phase II, 1	76.5	105.0	49.0	60.5	64.0	68.0	Negative	---
II, 2	79.5	81.0	58.0	71.0	66.5	66.0	Negative	Negative
III, 1	78.0	103.5	78.0	70.0	84.0	67.5	Negative	Negative
III, 2	81.5	109.0	110.0	86.5	72.5	41.0	1+	---
IV	78.5	111.0	76.0	91.5	97.5	90.5	Negative	Negative
Case 44								
Phase II, 1	80.0	138.0	128.5	86.5	84.5	45.5	1+	---
II, 2	84.5	123.5	129.0	82.0	99.5	76.5	2+	0
III, 1	74.5	123.5	120.5	80.5	125.5	58.0	1+	Trace
III, 2	74.0	94.0	101.0	75.0	67.5	102.0	1+	1+
IV	75.5	85.5	98.0	76.5	85.0	72.0	3+	1+
Case 45								
Phase III, 1	81.5	80.5	103.0	104.0	87.5	47.5	Negative	Negative
III, 2	79.5	124.0	113.0	78.5	109.5	60.5	Negative	Negative
IV	78.0	157.5	63.5	88.0	111.5	65.5	2+	Trace
Case 46								
Phase III, 1	73.0	141.5	126.5	108.5	103.0	41.0	Negative	Negative
III, 2	83.5	152.5	126.5	114.5	100.0	53.0	1+	1+
IV	54.0	121.0	109.0	118.0	153.5	79.0	Trace	Negative
Case 47								
Phase III, 1	75.0	109.0	88.5	81.0	65.0	77.0	Negative	Negative
III, 2	68.0	87.0	51.5	80.5	90.5	44.0	Negative	Negative
IV	57.0	79.0	85.5	69.5	87.0	74.5	Negative	Negative
Case 48								
Phase II, 1	81.0	125.0	146.0	83.0	82.5	87.5	1+	---
II, 2	81.5	129.5	112.0	82.0	83.5	90.0	Trace	0
III, 1	75.0	91.5	108.5	94.5	58.0	67.0	Trace	---
III, 2	74.0	119.5	119.5	131.5	65.5	74.5	---	0
IV	72.0	116.0	92.0	82.5	73.0	68.0	1+	Negative
Case 49								
Phase III, 1	74.0	102.5	41.5	82.0	81.0	36.5	Negative	---
III, 2	78.5	93.0	103.5	74.5	80.0	34.5	Negative	Negative
IV	70.0	106.5	106.5	74.0	90.5	49.0	Negative	Negative
Case 50								
Phase III, 1	86.5	95.5	85.0	71.5	84.0	73.0	Negative	---
III, 2	99.0	109.0	111.0	84.5	90.5	73.5	Negative	Negative
IV	97.5	143.5	90.0	102.5	71.5	110.5	Negative	Negative
Case 59								
Phase III, 1	77.5	109.0	132.5	125.5	104.0	58.5	Negative	Negative
III, 2	72.0	120.0	118.0	103.0	99.5	87.5	Negative	Negative
IV	76.0	115.5	113.0	118.5	114.0	85.0	Negative	Negative

NOTE: In these standard glucose tolerance tests each person was given a challenge of 100 grams after an overnight fast. In Phases II and III there was no alteration of the institutional diet. In Phase IV each person was on a high carbohydrate diet for the 3 days prior to the test.

Table V. Carbohydrate intake within 4 hours preceding glucose challenge: 24 men, Milan, Phase III

Case number	Intake in grams	
	Mean	Range
01	88.7	60-109
04	97.6	63-123
05	93.9	40-159
08	115.1	55-242
10	73.1	50-110
11	112.0	85-150
20	90.6	48-180
21	66.0	30-103
23	81.4	39-137
26	94.8	19-165
28	70.9	35-103
29	66.9	19-138
33	108.3	79-162
36	64.9	25-120
42	60.6	15-130
43	93.8	50-139
44	72.4	44-148
45	72.8	42-119
46	74.8	25-149
47	106.1	50-159
48	82.5	50-99
49	94.9	64-127
50	106.6	79-188
59	60.1	35-103

Table VI. Clinical classification of study participants by specified tests: 24 men, Milan, Phases III and IV

Case number	Age	Race	Phase III Tests[a]		Phase IV Tests[b]	
			SGTT1	SGTT2	SGTT	CGTT
01	44	White	0	0	0	+
04	41	White	0	0	0	0
05	43	Negro	0	0	0	0
08	46	Negro	0	0	---	---
10	47	Negro	0	0	0	0
11	48	Negro	0	0	0	0
20	44	White	0	0	[c]0	0
21	42	White	+	[c]0	+	+
23	41	White	0	0	0	0
26	43	White	0	0	?	0
28	52	White	0	0	+	+
29	49	Negro	0	0	0	+
33	40	White	0	0	0	0
36	48	White	0	0	---	---
42	46	Negro	0	0	0	0
43	46	Negro	0	0	0	0
44	43	White	0	0	0	0
45	45	White	0	0	0	+
46	40	Negro	0	0	0	0
47	43	White	0	0	0	0
48	42	White	0	0	0	0
49	41	White	0	0	0	0
50	41	White	0	0	0	0
59	42	White	0	0	0	0

[a]Usual diet.

[b]High carbohydrate preparatory diet.

[c]The response curve was above 150 mg.% at 1 hour and 110 mg.% at 2 hours and would be classified as "probable diabetic" by Unger.

NOTE: The SGTT (standard glucose tolerance test) and CGTT (cortisone glucose tolerance test) are defined in the text. The criteria used are those of Fajans and Conn. For the SGTT, if the response curve was above 160 mg.% at 1 hour, 140 at 1½ and 120 at 2 hours the person was classified "diabetic" (+). Response curves lower than this but above 160, 135, and 110 mg.% at the same points were classified as "probable diabetic" (+). All others were classified as "not diabetic" (0). For the CGTT, levels above 160 mg.% at 1 hour and 140 mg.% at 2 hours were classified as diabetic (+) and there was no borderline class.

Table VII. Heights, weights, and changes in weight: 24 men, Milan, Phase III

Case number	Height in inches	Initial weight in pounds	Final weight in pounds	Change in weight in pounds
01	68 1/2	175	175	0
04	67 1/2	186	184	-2
05	67	160	163	+3
08	69 1/2	225	228	+3
10	68	164	160	-4
11	67	161	163	+2
20	67	190	-	-
21	68 1/2	160	157	-3
23	68 1/2	184	180	-4
26	69	263	275	+12
28	67 1/2	177	176	-1
29	68 1/2	144	142	-2
33	70	182	184	+2
36	68 1/2	150	154	+4
42	69 1/2	214	217	+3
43	68	172	169	-3
44	69 1/2	176	176	0
45	76	180	176	-4
46	68	178	-	-
47	68 1/2	167	165	-2
48	66 1/2	151	145	-6
49	73 1/2	172	169	-3
50	69 1/2	173	172	-1
59	69	180	180	0

APPENDIX I

TECHNICAL VARIABILITY OF BLOOD GLUCOSE DETERMINATION

In any study, the reliability of measurement is an essential ingredient. For the Milan study, all blood glucose determinations were made by the laboratory of the Diabetes Field Research Unit of the Diabetes and Arthritis Branch, Division of Chronic Diseases, Bureau of State Services, U.S. Public Health Service. This laboratory has measured all of the blood glucose specimens of the Health Examination Survey.

Blood specimens of about 3 ml. were collected at the prison in prelabeled B-D "Vacutainers" (3204x, formula 44) containing 30 mg. of sodium fluoride. These were packed on ice within 3 hours of collection and were shipped by air mail, special delivery to the laboratory in Boston. Previous studies by the Health Examination Survey on the effects of handling and shipping specimens had shown that these factors have no discernible effect on the measurement. Tests were made in duplicate by the Somogyi-Nelson macromethod and the results were averaged. Generally, the laboratory work was performed the day after the specimens were collected. During most of the study, the same two technicians made all of the determinations, one of them measuring specimens for case numbers 1 through 29 (it varied slightly) and the other measuring the remaining specimens. Thus, most of the Phase III specimens for any specific study person were measured by one laboratory technician.

There are several gauges on the reliability of measurement during Phase III. The crudest is the weekly average for all specimens taken before challenge. Omitting case number 08, who came late into the study, and taking the value for the replicate week in the few instances where a specimen was missing for a given week, the average level before challenge varied from a low of 78.7 mg.% to a high of 88.7 mg.%. There was no indication of any trend with time in this average.

Another gauge is the difference between specimens taken a week apart on the same individual after an overnight fast. This yielded a "within-person" standard deviation of 6.5 mg.%. As already noted, these two specimens were almost always measured by the same technician. An unknown part of the variation represents the biological variation of fasting blood glucose levels. The remainder is the technical variability for a single technician in two laboratory "runs."

The third gauge is given by the various control specimens and standards measured by each technician as part of the routine of each laboratory "run". These are primarily working devices for uncovering obvious laboratory aberrations. On the standard 100 (a concentration of 100 mg.% of glucose dissolved in water), technician A averaged 99.9 mg.% during Phase III and technician B averaged 99.8 mg.%. On the standard 200, they averaged 197.3 and 198.5 mg.%, respectively. The other laboratory controls are somewhat better indicators of technical variability. On the serum control, technician A averaged 1.12 mg.% lower than technician B. On the blood control pool, she averaged 0.56 mg.% higher. If half of the squared difference between measurements made each week is averaged, the figure which results can be designated as total technician variability (within the same run). This average was 17.6 and 14.9 mg.% for blood and serum controls, respectively, or standard deviations of 4.2 and 3.9. Besides within-technician variability, these figures include a component of between-technician variability and between-run variability.

This will obviously be an underestimate of technician variability. As a more accurate gauge, a series of control specimens was introduced at the prison each week. This was done by taking replicate specimens in sequence from case numbers 46 and 42, and relabeling the blind duplicate with case numbers 12 and 14, respectively. Since these were bonafide case numbers used in Phase I, they could not be identified by the laboratory technicians as control specimens. Each week two specimens were sent to the laboratory for case number 12 (one for before challenge and one for 1 hour after challenge) and two for case number 14. Except for accidental losses, then, there were four pairs of replicate specimens in each laboratory series for a control. Because of the laboratory arrangements in force one specimen of each pair was measured by one technician, the replicate specimen of the pair by the other technician. Altogether there were 53 such comparisons from Phase III.

On the average, technician A measured the specimens 1.3 mg.% higher than technician B. Differences between replicate measurements ranged from 0.0 to 15.5 mg.%. This included an unknown variability arising

from field errors and differences in the handling of the specimens, a slight average difference between the level at which the two technicians customarily measured, between-technician and between-run variability, as well as the "pure" variability of the technicians. The total technical variability was 5.4 mg.%. It probably represents an overstatement of the technical variability in our study comparison, since in most cases the same technician measured nearly all the specimens from a specific examinee. The set of specimens averaged quite close to a level of 100 mg.%. For the specimens taken after challenge, the variability was greater than for the specimens taken before challenge, which accords with the usual experience that technician variability rises with the level of the specimen measured. The set of blind replicates which are most comparable in general level with the regular laboratory controls were those taken 1 hour after challenge from case number 46. These yield a figure of 6.6 mg.% as compared with an estimate from the laboratory control specimens of 4.0 mg.%.

Another series of blind replicates was obtained (from other specimens) by running aliquots from 25 specimens on an autoanalyzer at Ann Arbor to compare with regular determinations made at Boston during weeks 6-9 of Phase III. Besides showing that the autoanalyzer measured blood glucose concentration an average of 2.4 mg.% higher than did the technicians at the Boston laboratory, this comparison showed that during those weeks technician A was measuring 4.25 mg.% higher than technician B. The regular series of blind replicates for the same 4 weeks (an entirely different series of specimens) indicated an average technician difference of 4.23 mg.%. The almost exact agreement is, of course, quite accidental, but it does argue for the reliability of the control series introduced into the trials.

One final gauge may be mentioned. As an experiment, aliquots were drawn from one of the study participants (case number 59) during the course of Phase III. They were given a dummy case number (13), frozen, and retained frozen until after the study was completed. Twelve weeks after the end of Phase III they were thawed and shipped to the Boston laboratory for determination. If this process introduced no serious artifacts into the measurement, this series might uncover any laboratory drift that might have occurred during the study. All told, there were 24 specimens in this series (a pair of specimens for each of 12 weeks in Phase III). Twenty-two of these specimens were measured by the same technician (technician A) both on the original aliquot and the frozen aliquot.

For all specimens, except those for weeks 3, 4, and 14, the determinations on the frozen aliquots were higher than the original determinations. For 7 of the 12 weeks, the average difference between the original pair of determinations and the subsequent pair was less than 3 mg.%. The average difference was larger than this only for weeks 3, 4, 6, and 7, the largest being for week 7—8.25 mg.%. Admittedly, these data will not support a heavy load of inference but at the very least they can be said to give no evidence of a laboratory drift during Phase III.

If the differences between the original and frozen specimens can be regarded as representing the variability of technician A over the entire period of Phase III, the number to assign to that variability is 5.0 mg.%. The average level of these specimens is 113.6 mg.%.

It is possible to summarize the various indices of technical variability as follows. There are two measures of within-technician between-run variability, that from the fasting specimens for the same person and that from the frozen aliquots. The first standard deviation is 6.5 mg.% and the second is 5.0 mg.%. Since the first measure also includes a component arising from biological variation, it probably is an overstatement of the technical variability. Then there are the measures of variability from the control specimens and the blind replicates. While these came from measurements done the same week, they were derived from different laboratory runs and in addition include variation arising from technician differences. The first was 4.0 mg.% and the second 5.4 mg.%. It should be reiterated that in most instances specimens for the same person were measured by one technician through all of Phase III.

It seems reasonable to conclude from all the evidence that the effective technical variation for Phase III did not exceed 5 mg.%.

APPENDIX II

NOTES ON THE STATISTICS

The chief focus of the study was Phase III. During Phase III, each of 24 persons had each of 8 procedures performed in duplicate. Thus for any one procedure X, there will be two measures of blood sugar levels at 1 hour after challenge, x_1 and x_2.

$x_1 + x_2$ has a variance $(x_1 - x_2)^2 = d^2$

For one procedure undertaken on one person, x_1 and x_2 may be considered as statistically independent from y_1 and y_2 from any other procedure Y yielding blood glucose values for the same person.

The symmetry of these experimental arrangements leads to the nice result that comparing procedures for each person and pooling the results is exactly the same as comparing the mean levels for all 24 persons. Hence if $\bar{x}$ and $\bar{y}$ are the mean blood glucose levels for procedures X and Y then $\frac{\bar{x} - \bar{y}}{S_{\bar{x},\bar{y}}}$ has a Student's -t distribution with 48 degrees of freedom, where $S^2_{\bar{x},\bar{y}} = S^2_{\bar{x}} + S^2_{\bar{y}}$. $S^2_{\bar{x}}$ is, of course, $\frac{\sum d_i^2}{(2n)^2}$, where $d_i = x_1 - x_2$ for any one person and n is the number of persons.

In general, $S_{\bar{x}}$ is the value given in table 2 divided by $(48)^{1/2}$.

This test amounts to a comparison of differences between procedures against the "within-person, within-procedure" variability. An alternative procedure is to compare the average difference between procedures against the "between-person, between-procedure" variability. For this purpose, form the statistic $(x_1 + x_2) - (y_1 + y_2) = p$ for each person and compute the average of these values $\underline{p}$ for all 24 persons and the variance of these values $S^2_{\bar{p}} = \frac{\sum (p_i - \bar{p})^2}{n - 1}$ where n is the number of persons. Test, then, to see whether $\frac{\bar{p}}{(S^2_{\bar{p}}/n)^{1/2}}$ is significantly different from zero, using Student's -t distribution with 23 degrees of freedom. This was the procedure used for testing the urine glucose values. It was not used in testing blood glucose values, although it is a procedure that many analysts would prefer.

The procedure used for testing differences in blood glucose levels is specially vulnerable to the situation where a few persons show large differences while most persons show almost none. To check against this possibility, a sign test was used. Thus, where procedure X was being compared with procedure Y, $(x_1 + x_2) - (y_1 + y_2)$ will either be positive or negative. If 24 persons are compared, the null hypothesis calls for 12 differences to be positive. If 17 or more are positive, there are more positive values than would be expected by chance. Here, as elsewhere, tests are made at a level of 5%. In this instance, the test will always be a one-sided test, since it is intended as a check on conclusions already drawn from a test of differences in means.

The rank correlation computed in the text is the one proposed by Spearman. If x_i is the rank of the i^{th} person under procedure X and y_i is his rank under procedure Y and $d_i = x_i - y_i$ then $r = 1 - \frac{6\sum d_i^2}{n^3 - n}$, where n is the number of persons. $\underline{r}$ has the approximate variance of $S^2 = \frac{1 - r^2}{n - 2}$ and $\frac{r}{s}$ is distributed as Student's - $\underline{t}$ with $n - 2$ degrees of freedom. The test is a one-sided one.

Another test performed was for differences between variances. For this purpose a rough approximation was used by first computing the pooled variances for each person and testing these for homogeneity between persons and then performing a similar test for the pooled variance for each procedure. The statistic used was Hartley's M-statistic which is tabled in the Biometrika Tables for Statisticians, Volume I.

Two minor issues merit consideration. The first is the handling missing data. There were 5 occasions where a replicate measurement was not available for blood glucose levels before challenge and 4 where a

replicate measurement was not available for blood glucose levels after challenge. These are, of course, trivial omissions. In these cases the internal variance can be estimated to be the same as the average for the other persons tested by the specified procedure and all tests can be performed as if the missing information was present. In no case was the actual number of degrees of freedom less than 23 for any procedure and except for borderline tests the effect of assuming 24 degrees of freedom is negligible.

The second minor issue is the handling of abnormal data. There are several instances where the blood glucose level reported seemed unlikely for the person and the circumstances. There is no really satisfactory way of dealing with such cases. In general the best solution is to accept the data. But there are occasions when it seems completely inadmissible to accept the data. There were five such specimens in our series. Specifically:

In week 16, the fasting blood glucose for case number 23 was reported as 149.0 mg.%. This value was discarded and not replaced. In the 15 other cases where a blood specimen was taken before challenge from this person the level ranged from 62.5 to 101.0. The three other fasting specimens for this person were 72.5, 80.5, and 76.0 mg.%.

In week 15, case number 36 gave a fasting blood sugar level of 140.0 mg.% and a value 1 hour after challenge of 59.5 mg.%. These values were both discarded and not replaced. Both values are outside the range of other comparable values for this person and are very different from the paired values in week 16. It seems likely that their labels were reversed.

In week 15, case number 59 had values before and after challenge of 146.5 and 78.5 mg.%, respectively. Blind replicates for these same specimens were 144.0 and 83.5 mg.%. These values are both discarded for the same reasons as in the preceding case. The procedure in question was repeated on this person in week 18 and the results from this are used as replacements.

One last comment is in order. The study called for each person to be submitted successively to all factors under study. The major breach in this design was to divide the study participants into breakfast and lunch groups, with the expectation that varying the circumstances of challenge after breakfast would have the same effect as varying them after lunch. The results did not bear out these expectations.

It is always difficult to decide whether it is preferable to be able to make a limited statement with great assurance or to attempt to learn more at the risk of decreased precision. In this instance, more information was obtained than a rigid design would have allowed at the cost of a serious loss in neatness.

☆ U. S. GOVERNMENT PRINTING OFFICE : 1973 543-879/17

OUTLINE OF REPORT SERIES FOR VITAL AND HEALTH STATISTICS

Public Health Service Publication No. 1000

SERIES 1-4. GENERAL SERIES. Program descriptions, methodological research, and analytical studies of vital and health statistics. Earlier reports of this kind have appeared in "Vital Statistics–Special Reports" and in "Health Statistics from the National Health Survey," Series A and D, PHS Publication No. 584.

Series 1: Programs and collection procedures.–Reports which describe the general programs of the National Center for Health Statistics and its offices and divisions, data collection methods used, definitions, and other material necessary for understanding of the technical characteristics of published data.

Series 2: Data evaluation and methods research.–Studies of new statistical methodology including: experimental tests of new survey methods, studies of vital statistics collection methods, new analytical techniques, objective evaluations of reliability of collected data, contributions to statistical theory.

Series 3: Analytical Studies.–This series comprises reports presenting analytical or interpretive studies based on vital and health statistics.

Series 4: Documents and committee reports.–Final reports of major committees concerned with vital and health statistics and documents such as recommended model vital registration laws and revised birth and death certificates.

SERIES 10-12. DATA FROM THE NATIONAL HEALTH SURVEY

Earlier reports of the kind appearing in Series 10 have been issued as "Health Statistics from the National Health Survey," Series B and C, PHS Publication No. 584.

Series 10: Statistics on illness, accidental injuries, disability, use of hospital, medical, dental, and other services, and other health-related topics, based on data collected in the continuing National Health Interview Survey.

Series 11: Data from the Health Examination Survey based on the direct examination, testing, and measurement of national samples of the population of the United States, including the medically defined prevalence of specific diseases, and distributions of the population with respect to various physical and physiological measurements.

Series 12: Data from the Health Records Survey relating to the health characteristics of persons in institutions, and on hospital, medical nursing, and personal care received, based on national samples of establishments providing these services and samples of the residents of patients, or of records of the establishments.

SERIES 20-23. DATA FROM THE NATIONAL VITAL STATISTICS SYSTEM

Earlier reports of this kind have been issued in "Vital Statistics–Special Reports."

Series 20: Various reports on mortality, tabulations by cause of death, age, etc., time series of rates, data for geographic areas, States, cities, etc.–other than as included in annual or monthly reports.

Series 21: Data on natality such as birth by age of mother, birth order, geographic areas, States, cities, time series of rates, etc.–compilations of data not included in the regular annual volumes or monthly reports.

Series 22: Data on marriage and divorce by various demographic factors, geographic areas, etc.–other than that included in annual or monthly reports.

Series 23: Data from the program of sample surveys related to vital records. The subjects being covered in these surveys are varied including topics such as mortality by socioeconomic classes, hospitalization in the last year of life, X-ray exposure during pregnancy, etc.

Catalog Card

U.S. National Center for Health Statistics

The one-hour oral glucose tolerance test. Response of middle-aged men to 100-gram and 50-gram doses of glucose given fasting and 1, 2, and 3 hours after meal. Washington, U.S. Department of Health, Education, and Welfare. Public Health Service, 1963.

34 p. diagrs., tables. 27cm. (*Its* Vital and Health Statistics, Series 2, no. 3) U.S. Public Health Service. Publication no. 1000, Series 2, no. 3

1. Glucose metabolism. 2. Medical research - Methodology. I. Title. II. Title: Glucose tolerance test. III. Series: U.S. Public Health Service. Publication no. 1000, Series 2, no. 3.

Cataloged by Department of Health, Education, and Welfare Library.

DHEW Publication No. (HRA) 74-1271
Series 2-No. 3

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

Health Resources Administration

5600 Fishers Lane
Rockville, Maryland 20852

OFFICIAL BUSINESS
Penalty for Private Use $300