CARBOHYDRATE TOLERANCE IN THE NON-PREGNANT NATAL INDIAN FEMALE*

MORRIS NOTELOVITZ,† M.B., B.CH., M.D. (RAND), M.R.C.O.G., *Principal Gynaecologist and Obstetrician and Senior Lecturer at the University of Natal and King Edward VIII Hospital, Durban*

It has been stated[1] that 'the ease and accuracy with which the blood sugar can be measured may have led to an unwarranted pre-occupation with carbohydrate in the study of the metabolic disturbance of diabetes mellitus; derangements of lipid and protein metabolism are no less conspicuous, and perhaps more closely related to the central chemical fault... Nevertheless, in the diagnosis and clinical control of the diabetic patient, the bloodsugar is the principal guide and looks like remaining so for some time.'

Since the prevalence of diabetes (as measured by impaired glucose tolerance) is governed by differences in diet and other environmental factors, and as standards of normal tolerance are not necessarily applicable to populations of different countries,[2,3] it was considered necessary to evaluate 'normal' carbohydrate tolerance in the Natal Indian female, all previous studies having been based on 'normal' values obtained in other racial groups. The scope of this investigation was further designed to incorporate a study of the effect of age, parity, obesity, religion and hospitalization on the interpretation of 'normal' glucose tolerance.

METHOD OF STUDY

A glucose-tolerance test should exhibit maximum sensitivity (i.e. the ability to identify abnormal glucose tolerance), with specificity (the ability to identify correctly a person having normal glucose tolerance). In addition, it should be reproducible, easy to interpret and of practical value for use on a large scale. Of the many tests available, the '100-G 2-hour glucose tolerance test' was chosen because it met most of the requirements listed above.[4-7]

After an overnight fast, patients were given 100 G of glucose (dissolved in approximately 200 ml. of orange-flavoured water) to drink within 2 - 3 minutes. Venous blood and freshly voided specimens of urine were obtained in the fasting state and 2 hours after ingestion of the glucose. The blood glucose was assayed within 4 hours, all estimations being performed by the same technician in the Department of Gynaecology and Obstetrics, University of Natal, Durban. The degree of glycosuria was measured quantitatively, using glucose-oxidase paper (Tes-tape).

Blood-glucose Estimation

Departmental blood-sugar determination. The technique employed was based on methods described by Herbert and Bourne[8] and King.[9] In principle, the test involves the isotonic precipitation of proteins to prevent reducing substances in the red cells from contaminating the protein-free filtrate. Hot alkaline reduction of the cupric ion by glucose is the basic procedure, the degree being measured by the reduction of phosphomolybdic acid to 'molybdenum blue' by the formed cuprous ions. The 'molybdenum blue' is measured colorimetrically. This method is representative of total reducing substances in the blood.

'Laboratory' blood-sugar determinations. The blood-sugar samples determined by the Central Laboratory at King Edward VIII Hospital were processed on the Technicon Auto-analyzer,[10] the glucose being determined by a procedure utilizing the potassium-ferricyanide—potassium-ferrocyanide oxidation reduction reaction. The results of this technique reflect 'true' glucose levels.

MATERIAL

The Natal Indian females who formed the basis of this study were all of the same socio-economic class, differing only in their religious affiliation. A total of 268 women was studied.

Non-pregnant Outpatient Controls

The normal non-pregnant controls comprised healthy ambulatory Indian females in the childbearing age-group (16 - 45 years), who had accompanied patients to the consulting rooms. They totalled 200 in number and were divided into 4 subgroups according to their parity and family history of diabetes. All patients with a history of unexplained stillbirths and neonatal deaths, progressive increase in size of infants and symptoms suggestive of diabetes were excluded.

Hospitalized Patients

A further group of 68 non-pregnant Indian patients were investigated and comprised those patients who had been admitted to the professorial gynaecologicial wards during the month of October 1963, and who had had no signs or symptoms suggestive of diabetes.

RESULTS

The results of the above study are presented in relationship to the known influence of a family history of diabetes, age, parity and weight on carbohydrate tolerance. The role of the religious differences of the patients tested, the method of blood-sugar analysis and the effect of hospitalization on glucose tolerance were similarly analysed. The significance of glycosuria is presented separately.

Glucose Tolerance

Normal controls. The patients analysed in this category comprised Natal Indian females, in the childbearing age-group, who had had no history suggestive of diabetes and who were equally distributed as regards parity and age. A total of 100 patients was studied, and the results are reflected in Table I.

TABLE I. GLUCOSE TOLERANCE IN 100 NORMAL NON-PREGNANT NATAL INDIAN FEMALES

	Blood-sugar level (mg./100 ml.)	
	Fasting	*2-hour*
Mean	83·57	99·73
Variance	280·30	364·20
SD	16·74	19·10
Normal range (mean ± 2SD)	50·09 - 117·05	61·53 - 137·93

*Date received: 20 May 1968.

†Present address: Principal Gynaecologist and Obstetrician and Head of Department, Addington Hospital, Durban.

Thus, based on the formula of the mean plus or minus 2 standard deviations, the normal venous fasting blood-sugar value (Herbert and Bourne) for the non-pregnant Natal Indian diabetic was found to range between 50 and 120 mg./100 ml. and the 2-hour postprandial blood-sugar level between 60 and 140 mg./100 ml. Utilizing 140 mg./100 ml. as the upper limit of the normal, 4 patients (4%) would have been classified as diabetic. The actual values obtained in these patients were 144, 142, 154 and 140 mg./100 ml.

Family history. To ascertain the effect (if any) of a family history of diabetes on glucose tolerance, a further group of 100 Natal Indians were studied. These patients had a positive family history of diabetes but were equal in all other respects to the previous group.

Only the 2-hour postprandial values of these 2 groups were compared, and it was found that patients with a positive family history had a mean blood-sugar level of 114·34 mg./100 ml. compared with 99·73 mg./100 ml. in the control group. The difference was found to be statistically significant ($p = <0·01$) (see Table II).

TABLE II. COMPARISON OF MEAN 2-HOUR POSTGLUCOSE BLOOD-SUGAR LEVELS IN CONTROL PATIENTS WITH AND WITHOUT FAMILY HISTORIES OF DIABETES

	Blood-sugar level (mg./100 ml.)	
	Without family history	*With family history*
Sample tested	100	100
Mean	99·73	114·34
SD	19·10	40·97
Variance	364·2	1,668·51
't'-test for significance	$p = <0·01$	

Furthermore, no fewer than 12 patients in the positive history group had 2-hour blood-sugar values of 140 mg./100 ml. or more, as opposed to only 4 in the negative history group. This difference was also found to be significant when analysed by the chi-square test ($p = <0·05$; chi-square = 5·62).

Age. Using the results obtained previously, the patients were then divided into 3 age-groups: less than 20, 20 - 30, and 30 - 40 years. As indicated in Table III, there was a progressive intolerance to glucose with age. Only the 2-hour postprandial values were analysed statistically, and the difference between the age-groups was found to be significant at the 5% level.

TABLE III. COMPARISON OF MEAN 2-HOUR POSTPRANDIAL BLOOD-SUGAR LEVEL IN 3 AGE-GROUPS ANALYSED BY THE 'F' TEST

	Age-group		
	20 years	*20 - 30 years*	*30 - 40 years*
Mean	95·95	105·51	112·20
SD	17·00	34·36	22·46
Variance	289·57	1,181·12	504·25
'f' test for significance	$p = <0·05$		

Parity. The influence of parity was next assessed. Reference to Table IV demonstrates that increasing parity results in a significant lowering of carbohydrate tolerance. The differences of the mean 2-hour values alone, were analysed.

TABLE IV. COMPARISON OF MEAN 2-HOUR POSTGLUCOSE BLOOD-SUGAR VALUES IN NULLIPAROUS, PAROUS AND GRANDMULTIPAROUS PATIENTS

	Parity		
	0	*1 - 4*	*5 - 10*
Mean	99·73	109·34	111·25
SD	19·10	27·1	37·53
Variance	364·2	734·63	1,408·0
'f' test for significance	$p = <0·01$		

Weight. To determine the effect of obesity on glucose tolerance the patients were then divided into 4 groups—those weighing below 100 lb.; 100 - 130 lb.; 130 - 160 lb. and more than 160 lb. It was possible to classify all patients into the above groups since they had medium frames and their height seldom varied by more than 2 inches, the mean height being 5 ft. 1 in. Based on the Metropolitan Life Insurance Company's figures,[1] adult women in this category should weigh between 104 and 116 lb., while the upper value for women with large frames extends to 128 lb. Reference to Table V shows a progressive intolerance to glucose with increasing weight, which is statistically significant at the 1% level when analysed by the 'f' test.

TABLE V. EFFECT OF OBESITY ON MEAN 2-HOUR POSTGLUCOSE BLOOD-SUGAR VALUE

	Weight			
	100 lb.	*100-130 lb.*	*130-160 lb.*	*160 lb.*
Mean	93·48	104·53	119·18	114·84
SD	18·19	30·80	57·50	22·22
Variance	330·79	948·83	3,306·51	493·79
'f' test for significance	$p = <0·01$			

Hospitalization. Glucose tolerance was studied in 68 non-pregnant Indian females who had been admitted to the professorial gynaecological ward and who had had no signs or symptoms suggestive of diabetes. Since the average age of these patients was 35·7 years, the effect of hospitalization on glucose tolerance was compared with the 30 - 40-year 'outpatient' control group. No statistically significant difference could be demonstrated between the values obtained in the fasting and 2-hour postglucose blood levels (see Table VI).

TABLE VI. EFFECT OF HOSPITALIZATION ON GLUCOSE TOLERANCE

	Fasting values		*2-hour values*	
	Hospitalized controls	*Outpatient controls*	*Hospitalized controls*	*Outpatient controls*
No. of patients	68	65	115·70	112·20
Mean	91·75	90·70	46·59	22·46
SD	24·86	16·66	2,170·97	504·25
Variance	618·12	277·77		
't' test for significance	$p = >0·05$		$p = >0·05$	

However, there were more patients among the hospitalized 'controls' with 2-hour blood-sugar values above 140 mg./100 ml. than outpatient controls—16·2% and 10·8%, respectively. This difference is statistically significant (see Table VII).

TABLE VII. EFFECT OF HOSPITALIZATION ON GLUCOSE TOLERANCE

Controls	*No.*	*No. with values >140 mg./100 ml.*	*X^2 test for significance*
Outpatient	65	7 or 10·8%	$X^2 = 0·633$
Hospitalized	68	11 or 16·2%	$p = <0·05$

Method of blood-sugar determination. To ascertain whether any significant difference existed between the Herbert and Bourne and the Auto-analyzer methods of blood-sugar analysis, 100 glucose-tolerance tests were assayed by both techniques and the results compared. Whereas no statistical difference existed when the fasting blood-sugar samples were compared ($p = >0{\cdot}05$), the higher levels recorded by the modified Folin-Wu method after the ingestion of glucose was significant ($p = <0{\cdot}05$) (Table VIII).

TABLE VIII. COMPARISON OF METHOD OF BLOOD-SUGAR ANALYSIS IN 100 PATIENTS

	Fasting values		*Postglucose values*	
	Auto-analyzer	*Herbert & Bourne*	*Auto-analyzer*	*Herbert & Bourne*
Mean	81·78	82·92	103·24	110·07
SD	10·95	11·62	23·73	22·65
Variance	120·25	134·9	562·50	510·80
't' test for significance	$p = >0{\cdot}05$		$p = <0{\cdot}05$	

Religion. Patients with 2-hour postprandial blood-sugar levels above 140 mg./100 ml. or more were studied to establish whether any one of the following religious groups were more prone to the development of disordered carbohydrate metabolism. Thus of the 166 non-pregnant controls who were included in this aspect of the study, 68 were Moslem, 52 Tamil and 46 Hindu. The percentage of abnormal glucose-tolerance curves was 8·8%, 13·4% and 0·0%, respectively. Although the numbers are small, the differences when subjected to statistical analysis were found to be significant ($p = <0{\cdot}05$) (Table IX).

TABLE IX. RELIGION AND INCIDENCE OF ABNORMAL GLUCOSE TOLERANCE

Religion	*Number*	*Values above 140 mg./100 ml.*	X^2
Moslem	68	6 or 8·8%	$X^2 = 6{\cdot}29$
Tamil	52	7 or 13·4%	$p = <0{\cdot}05$
Hindu	46	0 or 0·0%	

Glycosuria

Of the 200 outpatient controls studied, only 4 had evidence of glycosuria 2 hours after the ingestion of 100 G of glucose (Tables X and XI). The incidence of glycosuria is therefore 2%. The results of a similar study in hospitalized patients showed an incidence of 20·5% (14 out of 68 patients tested). This difference is statistically highly significant ($p = <0{\cdot}001$).

TABLE X. INCIDENCE OF GLYCOSURIA IN NON-PREGNANT NATAL INDIAN FEMALES

Patients tested	*No. tested*	*Glycosuric %*	*% of glycosurics with positive GTT*
Outpatients	200	2·0 (4)	75·0 (3)
Hospitalized	68	20·5 (14)	42·8 (6)

TABLE XI. INCIDENCE OF ABNORMAL GLUCOSE TOLERANCE IN NON-PREGNANT NATAL INDIAN FEMALES: GLYCOSURIC SURVEY COMPARED WITH 2-HOUR BLOOD-SUGAR LEVELS

Patients tested	*No. tested*	*Glycosurics with abnormal GTT (A)*	*Apparent abnormal glucose tolerance*	*Aglycosurics with abnormal GTT (B)*	*True incidence of abnormality (A + B)*
Outpatients	200	3	1·5%	14	8·5%
Hospitalized	68	6	8·8%	4	14·6%

The patients with glycosuria were then analysed to determine the incidence of abnormal carbohydrate curves, and it was found that 75% of the outpatients (3/4) and 42·8% of the hospitalized patients (6/14) had positive curves.

Based on these results, the incidence of diabetes was calculated—thus the outpatient controls had an incidence of 1·5% (3/200), while 8·8% (6/68) of those in hospital had diabetic curves. When the blood-sugar levels of all the patients were analysed, however, it was found that a further 14 patients in the control group had results of 140 mg./100 ml. or higher, while the same was true of 4 of the hospitalized group. These patients were aglycosuric. Therefore the corrected incidence of diabetes in the control group is 8·5% (3 + 14 out of 200) and in the hospitalized group 14·6% (6 + 4 out of 68).

Closer scrutiny of the blood-sugar levels in the aglycosuric subjects showed that in the outpatient group, only one had a moderately elevated figure (190 mg./100 ml.), all the others varying between 140 and 150 mg./100 ml. Of the 4 hospitalized aglycosurics, levels of 275, 292 and 336 mg./100 ml. were recorded—evidence of raised renal thresholds.

DISCUSSION

Although the oral glucose-tolerance test is usually accepted as the clinical *sine qua non* for the diagnosis of diabetes, individual interpretations are often notoriously variable. Consequently, evaluation of normal glucose tolerance will be incomplete without consideration of the following factors.

Dietary Preparation Before Test

Conn[32] first described the necessity of dietary preparation for the glucose-tolerance test and recommended that all persons should have a diet of 300 G of carbohydrate for at least 3 days before the test. Krall[33] maintains that insistence upon an effective diet has been overemphasized and that only quasi-starvation diets with very sparse carbohydrate content will give falsely abnormal glucose-tolerance curves. Similar conclusions were reached by Wilkerson *et al.*[34] who found that no significant alterations in glucose tolerance were observed until diets were decreased to below 150 G/day—a figure far below the average daily eating pattern.

The daily diet of the Natal Indian consists mainly of carbohydrate (rice), while the consumption of sugar averages 35 - 50 kg. *per annum*[35]—an intake similar to that of people in Britain. Consequently we consider dietary preparation to be satisfactory, provided that an unrestricted diet has been eaten 3 - 4 days before testing. Insistence upon a positive history of an adequate dietary intake from patients in the series thus lessened the possibility of obtaining false glucose-tolerance curves.

Effect of Recent Food in Glucose Tolerance

Hamman and Hirschman[36] demonstrated that if carbohydrate is administered orally in 2 doses, the blood sugar rises less after the second dose. This phenomenon was confirmed by Staub[37] and is today known as the 'Staub effect'.

The concept that prior ingestion of carbohydrate interferes with glucose tolerance has recently been challenged

by Hayner *et al.*[18,19] They have shown that glucose tolerance (as judged by the one-hour glucose response) is as reproducible and efficient postprandially as in the fasting state.

Similar studies were conducted in Uruguay and Venezuela[2] and it was shown that the mean 2-hour blood-glucose value of those who had fasted more than 4 hours was 99 and 102 mg./100 ml., respectively, while the mean level of those who had fasted only 2 - 4 hours was 91 and 98 mg./100 ml.

Therefore, although all our patients were instructed to fast overnight for at least 12 hours, the consumption of small amounts of carbohydrate (particularly in the out-patients) would probably have had little effect on the result of the glucose-tolerance test. Patients who admitted to having had breakfast were excluded from the series.

Oral Glucose Tolerance and Dose

Maclean and De Wesselow[20] maintain that it is not necessary to adjust the dose of glucose to the individual's weight, as 25 G of glucose elicited the maximal glycaemic response, larger amounts tending to prolong the period during which the blood-sugar level remained elevated, rather than increasing the actual height of the sugar curve.

Jackson[21] compared the results of the 50-G test with a dosage based on 1·75 G/kg. of ideal body-weight and found that there was a distinct delay in the fall of the blood-sugar level on the 'ideal body-weight' test. Similar results were obtained by West *et al.*[22] who compared the administration of 0·8 G/kg. with 1·6 G/kg. of ideal body-weight, and obtained values which were 21 mg./100 ml. higher with the bigger dosage.

Although Wilder[23] observed little difference in response to glucose tolerance when the oral dose was varied between 50 and 150 G, the consensus of opinion is that revision of one's criteria is necessary when larger loading-doses are used. The reason for selection of the larger dose —100 G—in the present study is discussed later.

Type of Carbohydrate

An investigation in 1964[22] highlighted the importance of the type of carbohydrate used in the oral glucose-tolerance test. West *et al.*[22] compared glucose tolerance following breakfast and a glucose load, both of which contained the equivalent of 75 G of carbohydrate, and found that the blood-sugar levels were significantly higher following glucose administration. Thus the 2-hour blood-glucose levels were 21 mg./100 ml. higher after 75 G of oral glucose than after a breakfast containing 75 G of carbohydrate. Nevertheless, these authors concluded that it was better to employ a test-meal for screening, as it presented a more physiological challenge and was, in addition, more palatable and convenient.

This method would not be practicable in our unit, since the patients are largely illiterate and unreliable, and it is therefore doubtful whether the prescribed breakfast would be consumed at the requested time. Furthermore, extra allowance would have to be made for the consumption of calories since most of our patients have to walk and travel long distances when attending clinics.

We therefore find it practical to administer a glucose loading-dose as described earlier.

Duration of Glucose-tolerance Test

Although the value of a glucose-loading test as a means of measuring carbohydrate tolerance was first reported by Jacobson[24] in 1913, there is still no universal agreement as to the ideal duration of a glucose-tolerance test.

Hayner *et al.*[19] and others[4] maintain that a single blood-sugar determination one hour after a carbohydrate load is as efficient for the detection of diabetes as a single 2-hour test. Similar views are expressed by Krall,[13] although he emphasizes that such a test is mainly of value as a screening device. In non-diabetics the one-hour postprandial blood-sugar level depends on the rapidity of absorption from the gastro-intestinal tract,[25] becoming extreme with the so-called 'dumping syndrome' following gastro-enterostomy and subtotal gastrectomy. Mosenthal and Barry[26] found that the height of the blood-sugar level at one hour varied so frequently (40%) that it could not be regarded as a reliable means of interpreting glucose tolerance.

The accuracy of a single assay at 2 hours, however, approaches that of a full glucose-tolerance test when the given criteria for interpretation of the test are used, e.g. Stewart and Robertson[5] had values of 97% and 99% for sensitivity and specificity, respectively, using a 2-hour blood-sugar level of 130 mg./100 ml. (Folin-Wu). Mosenthal and Barry[26] insist that the 2-hour blood-sugar level must be elevated in order for a glucose-tolerance test to indicate diminished ability to utilize carbohydrate, while Jackson[21] has recently stated that 'It is agreed that the two hour blood sugar level is the most important single figure in the assessment of tolerance for 50 grams of glucose and abnormalities of this figure particularly help to distinguish diabetics from normals'. The diagnostic value of the 2-hour postprandial glucose level has subsequently been endorsed by numerous other investigators.[4-7,27,28]

Despite the many variations of methods to determine altered glucose tolerance, the standard 3-hour glucose-tolerance test is still the most reliable in terms of reproducibility, sensitivity and specificity.[13,21] Five specimens of blood are tested and if any one figure is too high the curve is considered abnormal; if 2 or more figures are above these limits, the curve is said to be diabetic.[13] The only disadvantages are the number of blood samples that have to be withdrawn and the longer duration of the test.

The 2-hour 100-G glucose-tolerance test has been favoured in the obstetric unit at King Edward VIII Hospital because only 2 blood samples are needed, thereby requiring fewer analyses. The test is of shorter duration and it is simple to interpret. While the upper limit of normal chosen originally—140 mg./100 ml. (Herbert and Bourne) —is possibly higher than that required by some authors,[5,21,29] the larger loading-dose invariably compensates for this strict criterion.

Consequently, in a unit where the delivery rate exceeds 20,000 births *per annum*, we have a diagnostic procedure which is tailored to our needs without loss of efficiency and with a tendency towards false positives rather than false negatives.

Criteria for Assessing Glucose Tolerance

The criteria for the assessment of normal glucose tolerance are influenced by both the method of blood-sugar

assay and the source of the blood sample.

Estimation of blood sugar. Jackson[21] employs the Hagedorn-Jensen method of blood-sugar estimation, which, like that of Folin and Wu, measures the total reducing substances, thereby giving readings higher than 'true blood-sugar' methods.

Mosenthal and Barry[23] and others[30] have found that the variability in the amount of non-glucose-reducing substances in the blood approximates 20 mg./100 ml. Therefore a rough rule of thumb is to allow this difference between the true glucose and the higher Folin and Wu blood-sugar level.[26,30,31] Unfortunately this is not consistent, as Mosenthal[32] found that the difference was greater than 30 mg./100 ml. in 38% out of 200 consecutive cases, while Wilkerson *et al.*[30] recorded a standard deviation of 7·7 mg./100 ml.

Krall[12] has therefore concluded that 'true sugar values with venous blood are preferred to other methods of testing diagnostically because they are generally more dependable technically and at present there is greater understanding of result interpretation'.

The 'total reducing' method[8,9] of blood-sugar analysis was retained in our unit because a 'true' glucose method was not available as a routine procedure in the early stages of the present investigation, and consequently the diagnosis and control of all the diabetics were based on this method of assay. To maintain uniformity, similar methods were employed in assessing normal glucose tolerance in the 'control' groups.

It occurred to me that, provided a pure glucose solution was used, the difference between the fasting and 2-hour postprandial blood-sugar levels should be similar and independent of the method of assay, since the only caloric supplement during the test is glucose. To test this hypothesis, the blood samples of 100 glucose-tolerance tests were analysed by both the 'total' and the true blood-sugar techniques. Whereas no statistical difference existed when the fasting blood-sugar results were compared ($p = >0{\cdot}05$), the 2-hour postprandial values as determined by the modified Folin and Wu technique (Herbert and Bourne) were significantly higher ($p = <0{\cdot}05$) than the 'true' blood-sugar method, although the difference between the means was only 6·83 mg./100 ml. This result therefore confirms the observation that the type of carbohydrate used in the oral glucose-tolerance test can alter the result appreciably, and it is therefore important that the quality of the test material be established and standardized before interpreting carbohydrate tolerance.

The interpretation of a glucose-tolerance curve is also dependent upon the source of the blood sample, as capillary blood—except in the fasting state—contains more sugar than venous blood. Thus, at the Joslin Clinic,[33] the capillary blood-sugar level is estimated at an average of 30 mg./100 ml. higher than venous blood, while at upper blood-sugar levels an even greater difference is apparent. Others[21,33] maintain that the peak difference between arterial and venous glucose may be between 20 and 70 mg./100 ml.

Jackson[21] prefers to use capillary blood, as it saves repeated venepuncture and is more likely to show the very early hypoglycaemic phase that characterizes the 'lag storage curve'. The micro- (capillary) technique is also employed at the Joslin Clinic, but only for screening purposes, venous blood being preferred for diagnostic measurement.

The results of the present investigation are based entirely on venous blood sampling.

It has been shown[34] that the excess pressure of a tourniquet produces considerable fluctuation in the venous blood-sugar level. It has been suggested[26] that the gentle application of the patient's free hand 2 inches above the antecubital vein should rather be employed. A similar procedure was adopted by me.

Normal Glucose-tolerance Curves

Normal values for glucose tolerance have been established by adding 2 standard deviations to the mean value of blood glucose obtained at ½- or 1-hourly intervals.[2,27,29] Although this method is not necessarily 'a logical or appropriate one',[27] Jackson,[21] by calculating percentiles from some of his figures, found that they tallied fairly closely with his SD methods, provided that the distribution curves of the blood-sugar levels at different times were of a Gaussian or 'normal' distribution. O'Sullivan and Mahan[29] found that the resulting number of people who yielded positive results to the same test approximated the presence of diabetes in a community. They preferred this conservative approach (mean + 2 SD) because by lowering the criteria (mean + 1 SD) numerically more people would be alerted for the benefit of a few true potential diabetics; making the criteria more strict (mean + 3D) would result in many prediabetics being missed. To illustrate this point further, West and Kalbfleisch[2] detected an apparent prevalence of diabetes in 6·8% of Uruguayan adults when the criterion of abnormality was set at 150 mg./100 ml., whereas if the 2-hour blood-sugar level was lowered to 120 mg./100 ml., 13·1% would have been classified as diabetic.

I therefore felt justified in adopting the formula of the mean plus 2 standard deviations to calculate the normal values of tolerance to a loading dose of 100 G of glucose in young, healthy, non-pregnant Natal Indian females. The values obtained (fasting level 50 - 120 mg./100 ml. and a 2-hour postglucose level of 60 - 140 mg./100 ml.) are slightly higher than those determined by some authors (Table XII), but correlated well with the levels of normality we had adopted before this study, and which were based on glucose-tolerance tests performed in normal Caucasian populations. Thus it would appear that the young Natal Indian female responds in a similar fashion to a loading dose of carbohydrate, and that glucose-tolerance curves

TABLE XII. CRITERIA FOR INTERPRETATION OF NORMAL GLUCOSE TOLERANCE*

Test-dose method	*Author*	*Fasting level*	*1-hour post-prandial*	*2-hour post-prandial*	*3-hour post-prandial*
100-G venous Somogyi-Nelson	Fajans (1960)	100	160	110	
	Mosenthal and Barry (1950)	100	150	100	
	Wilkerson *et al.* (1960)	100	170	120	
	Kroll (1965)	110	150	120	110
100-G venous Folin-Wu	Mosenthal and Barry (1950)	120	170	120	
	O'Sullivan and Mahan (1964)	110	170	120	110
	King Edward VIII Hospital: Natal Indian female	120		140	
50-G capillary Folin-Wu	Jackson (1964)	120	200	140	120
	Stewart and Robertson (1963)	120	180	140	

* Figures indicate upper levels of normal blood sugar expressed in mg./100 ml.

reported in the literature are applicable to this group as well.

It is pertinent to note, however, that whereas refined statistical techniques are of importance, careful consideration must also be given to the selection of the population studied.[29] Thus, large groups of unselected patients should be tested, bearing in mind factors such as age, weight, parity and family history.

Factors Affecting Glucose Tolerance

Family history of diabetes. As it is generally agreed that hereditary factors play an important role in the development of diabetes, it is reasonable to presume that glucose tolerance will be similarly affected.

Fajans and Conn[35] report a positive response of 25% in subjects with a diabetic history, compared with a 3 - 4% incidence in controls without diabetic heredity. Applying the same test methods to diabetic relatives, Jacobson *et al.*[36] report that 22% of them had impaired glucose tolerance, while in a similar study West[37] recorded impaired tolerance in 29% of subjects whose parents were diabetic.

Decreased glucose tolerance in people with family histories of diabetes has also been recorded when the intravenous glucose-tolerance test was used.[38,39]

To ascertain this feature in the Natal Indian female, 100 patients with a positive family history of diabetes were subjected to the 100-G oral glucose-tolerance test, and the 2-hour blood-sugar values were compared with those of a 'normal' control group. Thus the mean 2-hour blood-sugar values were found to be 114·34 mg./100 ml. and 99·73 mg./100 ml., respectively—a difference which is statistically significant ($p = <0{\cdot}01$). Furthermore, no fewer than 12 patients in the 'positive family history group' had 2-hour blood-sugar values of 140 mg./100 ml. or more, as opposed to only 4 patients in the 'negative history group'.

This study therefore confirms that glucose tolerance is affected in persons with family histories of diabetes and emphasizes the importance of (*a*) excluding patients with positive family histories when evaluating normal carbohydrate tolerance and, conversely, (*b*) subjecting all persons with a positive family history to a glucose-tolerance test, even though they may be asymptomatic at the time.

Age. A progressive deterioration of glucose tolerance with age was first recorded by Spence[40] in 1921. More recently, Jackson[21] and others[30,27,41,42] have demonstrated that the oral glucose-tolerance curve rises successively with each decade, the gradient varying between 10 and 13 mg./100 ml./decade.[39,41] Similar conclusions have been reached by studies based on the intravenous glucose-tolerance[43,44] and the intravenous tolbutamide test.[45] Two recent studies[26,46] have indicated that while an age gradient does exist for the 2-hour value of an oral glucose-tolerance test, it is a much more moderate slope than that of the one-hour value.

The decreased tolerance to sugar exhibited by the elderly has been explained on the basis of atrophy of the pancreas—a progressive decrease in pancreatic weight;[47] degeneration of islet cell blood-vessels,[48] decrease in the beta/alpha cell ratio[49] and a decrease in the immunoreactive insulin[48] have all been noted. Blotner[50] maintains that inactivity associated with bed rest is the prime factor.

Although all the patients in the present series were in the childbearing age-group (the youngest being 16 years and the oldest 46 years), a deterioration in glucose tolerance similar to that reported by Jackson[21] was observed. The differences between the mean 2-hour postprandial blood-sugar levels per decade are statistically significant ($p = <0{\cdot}05$). As these subjects were all ambulatory and in good health, it is apparent that increasing age (even in the young) does affect tolerance to glucose and adds weight to Jackson's suggestion that everyone is diabetic—some a little more so than others.

Parity. In 1956 Pyke[51] demonstrated that the incidence of diabetes in women rose with parity until the frequency in the most parous group was 7 times that of men. These observations have recently been confirmed by Fitzgerald and co-workers[52] who noted that, compared with nulliparae, diabetes is twice as common in women who have had 3 children and 6 times as common in those who have had 6 or more children. It would therefore be logical to conclude that repeated pregnancies reduced tolerance to glucose. Murphy[53] reported that glucose tolerance was depressed in 36 out of 50 women who had borne 10 or more children.

Lunell[38] investigated intravenous glucose tolerance in 2 groups of women of similar age and weight and concluded that parity had no significant influence on glucose tolerance. Jackson[21] also maintains that parity has no effect on glucose tolerance, yet perusal of his results shows an increasing number of diabetic curves with parity—8% of nulliparous women had diabetic curves compared with 22% in the para-1 - 5 group and 29% in those women who had had 6 - 9 infants. The number of diabetic curves in the para-6 - 9 group is significantly higher ($p = <0{\cdot}05$) than that in the nulliparous group. Their 55 grandmultiparae (para 10 or more) showed slight impairment of glucose tolerance, but their mean age was lower—47 years as compared with 53, 56 and 57 years in the other groups. These results may be compared with those reported by West and Kalbfleisch,[2] in which it is noted that half the women who had impaired glucose tolerance had had 5 or more full-term pregnancies.

Similar results were noted when the effect of parity on glucose tolerance was studied in the Natal Indian female. Thus a progressive impairment of glucose tolerance was found to develop with increasing parity; an observation which is not related to age, as the 3 groups were equally matched in this regard.

Obesity. The association between obesity and diabetes dates back to the observation of Lanceraux[54] in 1877. Various workers[55,56] have since found that approximately 60% of obese people have abnormal glucose tests, the impaired metabolism increasing in proportion to the duration rather than to the degree of obesity.[57] Newburger and Conn[58] have furthermore demonstrated that impaired glucose tolerance returned to normal after significant weight reduction.

More recently, several authors have confirmed the occurrence of impaired glucose tolerance in obese persons when using the oral[59,60] as well as the intravenous glucose-tolerance test.[61] Medley[62] studied an obese population by means of a prednisone-stressed intravenous glucose-tole-

rance test and showed that obese people have a small but statistically significant impairment of glucose tolerance when compared with a normal group. He concluded that obesity was not the cause, but rather a precipitating factor in those predisposed to diabetes, or that obesity was a consequence of the prediabetic state; an observation closely related to the concept that 'obese diabetics become obese because they are diabetic'.[63]

Tolerance in the Natal Indian was similarly affected by obesity. Thus the mean 2-hour postprandial values rose significantly with the increase in body-weight (Table V), and it is therefore suggested that in view of the high incidence of diabetes in this population group, obesity *per se* should be a valid indication for a glucose-tolerance test.

Religion. Although variation in glucose tolerance between populations of different countries and races has been well demonstrated, scant attention has been paid to variations in tolerance due to religious differences.[2] Fortunately, the Natal Indian affords an excellent opportunity for the study of this factor. Campbell[15] has noted that the incidence of diabetes is greater in the working-class Urdu-speaking Moslem than in the wealthier Gujurati Moslems and in the Indians of Hindu stock. Similar observations have been reported by workers in the Transvaal[64] and Kampala.[65] It has not yet been ascertained whether the increased prevalence in Moslems is due to their higher socio-economic status, their dietary differences, or their practice of intermarriage.

In East Pakistan 519 subjects were recently tested, and whereas the majority of these were Moslems, the prevalence of diabetes was slightly higher in the Hindus (2·1%) than in the Moslems (1·3%). This difference is not statistically significant.

The results of the present series (though based on very small numbers) indicate that of the 3 religious groups studied, the Tamil-speaking Indian is more liable to abnormal carbohydrate tolerance, followed by the Urdu-speaking Moslem. Of the 52 Tamilians, 13·4% had postprandial values of 140 mg./100 ml. or more, compared with 8·8% and 0% in the Moslems and Hindustani-speaking Hindus, respectively. These differences, when analysed by the X^2 formula, are significant.

Hospitalization. Since many normal values are based on the evaluation of investigations performed on hospitalized patients, it was decided to find whether glucose tolerance would differ between ambulatory controls, of similar age and parity, and patients hospitalized for conditions unrelated to diabetes. The results (Table XI) indicate that no statistical difference existed between the fasting and 2-hour blood-sugar levels when two such groups were studied, the fasting values being 91·75 and 90·70 mg./100 ml. and the postprandial levels 115·70 and 112·20 mg./100 ml. for the hospitalized and the ambulatory controls, respectively. However, although only slight differences in the mean values of the two groups could be demonstrated, it should be remembered that the groups tested were small and that analysis of individual results revealed more patients with abnormal glucose-tolerance tests in the hospitalized 'controls' than in a comparable 'outpatient' control group—16·2% and 10·8% respectively. This difference is statistically significant and serves to emphasize the importance of individual assessment of patients and the need to base normal values on healthy ambulatory patients.

Glycosuria. It has recently been stated[15,66] that renal glycosuria is very common in Natal Indian diabetics, while renal thresholds are uncommonly high. Two patients in the reported series had 2-hour postprandial blood-sugar levels of 380 and 480 mg./100 ml. without evidence of glycosuria.[15,66] Similar observations have been noted by Crombie[67] and others,[2,7,13,21,33,68] confirming that the absence of glycosuria does not exclude impairment of glucose tolerance. Furthermore, if reliance is placed upon the presence of glycosuria (fasting or postprandial) for screening of population groups, between 8 and 30% of diabetics will be missed.

The results of the present study indicate that whereas the 2% incidence of glycosuria in healthy ambulatory subjects is comparatively low (compared with 5 - 6% in a survey of glycosuria in Indians in the Transvaal), that of hospitalized patients is extremely high (20·5%). This difference may be explained in part by the fact that the hospitalized patients were older (mean age 33·6 years), and more parous (mean parity 5·5) than the outpatients (mean age 27·5 years, mean parity 3·8). Other contributory factors may be related to the observations of Blotner[50] that prolonged bed rest resulted in a lowering of the renal threshold, while the stress of the patient's particular condition probably acts in a similar fashion (*cf.* cortisone effect).

The presence of glycosuria in the non-pregnant Natal Indian is highly significant since the probability of it being due to diabetes is extremely high. Thus, 75% of the glycosurics in the outpatient series had diabetes (or prediabetes) while the same was true for 42·8% of the patients in hospital. I therefore firmly support the oft-quoted statement of Joslin[69] that 'if a patient has sugar in the urine, it is a safe rule to consider the diagnosis to be diabetes until the contrary is proved'. The same is not true of the pregnant subject.

Although this particular study was not designed to determine the incidence of diabetes in the population, analysis of the results has confirmed the reports of others,[2,7,21,28] that glycosuric surveys for this purpose yield fallacious results. Thus the incidence of diabetes in the Natal Indian female, if based on outpatient controls, is 1·5%. However, 14 of the patients with aglycosuria were found to have elevated postprandial blood-sugar levels, resulting in a corrected incidence of 8·5% (Table XI). Similar results were obtained in the hospital group—8·8% compared with 14·6%, respectively. Whereas the majority of the postprandial blood-sugar levels were only slightly elevated in the positive outpatient aglycosurics (140 - 150 mg./100 ml.), 3 patients in the hospital series had blood-sugar levels of 275, 292 and 336 mg./100 ml. without evidence of sugar in the urine, confirming Campbell's observation[15] of high renal thresholds in Natal Indians.

It may therefore be concluded that:

(*a*) There is a significant difference in the incidence of glycosuria in healthy ambulatory non-pregnant Natal Indian females when compared with hospitalized patients of the same race and socio-economic status.

(*b*) The presence of sugar in the urine of these patients is highly significant and always warrants further investigation for diabetes.

(*c*) Surveys to determine the incidence of diabetes in population groups should not be based on the presence of glycosuria, since elevated renal thresholds are common, as in the Natal Indian, and many aglycosuric diabetics will consequently be missed, thus producing fallacious results.

SUMMARY AND CONCLUSIONS

To determine the baseline for normal carbohydrate tolerance in the non-pregnant Natal Indian female, 268 patients in the childbearing age-group were subjected to the 100-G oral glucose-tolerance test.

Based on venous blood samples, the normal range (calculated by the mean plus 2 standard deviations) was found to vary between fasting values of 80 and 120 mg./100 ml., and 2-hour postglucose levels of 100 - 140 mg./100 ml. The blood-sugar samples were analysed by the methods described by Herbert and Bourne, and King, and are representative of 'total' reducing substances.

A family history of diabetes, increasing age and parity, and obesity were associated with a progressive decrease in carbohydrate tolerance. These factors constitute valid indications for a glucose-tolerance test in the Natal Indian.

This study confirmed that both the type of carbohydrate and the method of blood-sugar analysis can appreciably alter the result of glucose tolerance. Emphasis is placed on the need for the standardization of the method of glucose assay, and the material used.

A significant difference in the incidence of glycosuria among healthy ambulatory non-pregnant Natal Indians was found when compared with hospitalized patients of the same race and socio-economic status: 2% and 20·5%, respectively. The reason for this discrepancy is discussed and the need to base 'normal values' on healthy 'outpatient' subjects is stressed.

Whereas the presence of glycosuria in the non-pregnant Natal Indian female is highly suggestive of disturbed carbohydrate metabolism, aglycosuria does not exclude this possibility. Thus 8·5% of the total ambulatory control group were found to have abnormal carbohydrate-tolerance curves, as opposed to only 1·5% of those who presented with glycosuria. Investigations to determine the incidence of diabetes in population groups should not therefore be based solely on glycosuric surveys.

Evaluation of normal glucose tolerance is dependent upon the consideration of a number of variable factors such as the dietary preparation preceding the test, the effect of recent food on glucose tolerance, the loading dose of the test material, the type of carbohydrate used for this purpose and the duration of the test. These factors are discussed and the literature is briefly reviewed.

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