Studies in Methods of Investigating Carbohydrate

A. BILLIS and G. K. RASTOGI

The Metabolic Unit, Royal Victoria Hospital, Belfast

Received April 30, 1966

Summary. The carbohydrate tolerance of 50 control women and 101 women clinically suspected as "potentially" diabetic ("prediabetic") was investigated by the combined use of four tests; oral, intravenous, cortisone-glucose tolerance test and prednisone-glycosuria test. — Although the results confirm the view that none of the tests is adequately reliable, it seems that the intravenous and cortisone-GTTs qualify better for this purpose. — Thus, the intravenous G.T.T. showed remarkable reproducibility and a satisfactory relationship of impaired results with obstetrical histories strongly suggestive of potential diabetes, such as overweight babies and perinatal infant deaths. However, the test is difficult to perform and interpret properly and borderline results frequently occur. — Oral GTT often failed to disclose a presumably underlying abnormality in subjects with strong clinical histories and impaired intravenous G.T.T.s. — Cortisone-G.T.T. was shown to be more sensitive than the oral, and simpler than the intravenous test; it appears to be a suitable test for screening purpose in pregnancy. — Prednisone-glycosuria test disclosed a high proportion of false positive results, especially in subjects with renal glycosuria; it is therefore of doubtful diagnostic value in pregnancy. — Pregnancy seems to exert a progressive diabetogenic effect, more manifest in predisposed subjects; therefore tests are more likely to reveal abnormalities in third trimester. — Three women, all having impaired intravenous G.T.T.s, lost their babies in this pregnancy. It is suggested that early detection of potential diabetics and proper obstetrical care are of the utmost importance in lowering the perinatal mortality.

Etudes des méthodes d'investigation de la tolérance aux hydrates de carbone pendant la grossesse.

Résumé. On a étudié la tolérance aux hydrates de carbone de 50 sujets témoins et de 101 femmes cliniquement présumées diabétiques «potentielles» («prédiabétiques») par l'emploi combiné de quatre tests: le test de tolérance au glucose donné par voie orale, le test au glucose intra-veineux, le test de tolérance au glucose et à la cortisone associés, enfin le test de glycosurie à la prednisone. — Bien que les résultats confirment le point de vue selon lequel aucun de ces tests n'est tout à fait sûr, il semble que le G.T.T. intra-veineux et le G.T.T. à la cortisone soient les mieux appropriés. — Ainsi le G.T.T. intra-veineux a montré une reproductibilité remarquable et une relation satisfaisante entre les résultats anormaux et les histoires obstétricales suggérant fortement un diabète potentiel, telles que les enfants de poids élevé et les morts périnatales. Cependant le test est difficile à exécuter et à interpréter exactement et on a souvent des résultats qui sont à la limite. — Le G.T.T. oral a été souvent incapable de révéler une anomalie probablement latente chez des sujets ayant des histoires cliniques importantes et des G.T.T.s intra-veineux anormaux. — Le G.T.T. à la cortisone s'est montré plus sensible que le test oral et

plus simple que le test intra-veineux; il semble être un test approprié au screening pendant la grossesse. — Le test de glycosurie à la prednisone a révélé une grande proportion de faux résultats positifs, spécialement chez les sujets présentant une glycosurie rénale; il est donc d'une valeur diagnostique douteuse dans la grossesse. — La grossesse semble exercer un effet diabétogène progressif, plus manifeste chez les sujets prédisposés; en conséquence les tests ont plus de chance de révéler des anomalies pendant le troisième trimestre. — Trois femmes, ayant toutes des G.T.T.s intra-veineux anormaux, ont perdu leur bébé pendant la grossesse correspondante. Il est suggéré qu'une détection précoce des diabétiques potentielles et que des soins obstétricaux appropriés sont d'une importance capitale pour diminuer la mortalité périnatale.

Prüfung von Methoden zur Erfassung der Kohlenhydrattoleranz in der Schwangerschaft.

Zusammenfassung. Bei 50 Kontrollpersonen und 101 klinisch als „potentiell“ diabetisch verdächtigen („prädiabetischen“) Frauen wurde die Kohlenhydrattoleranz mit der kombinierten Anwendung von vier Testen untersucht: oraler, intravenöser, Cortison-Glukose-Toleranztest und Prednison-Harnzuckerausscheidungstest. — Obwohl die Ergebnisse die Ansicht bestätigen, daß keiner dieser Tests völlig zuverlässig ist, scheinen doch der intravenöse und Cortison-G.T.T. für diesen Zweck besser geeignet zu sein. — So zeigte der intravenöse G.T.T. eine erwünschtere Reproduzierbarkeit und eine befriedigende Beziehung zwischen den pathologischen Ergebnissen und den geburtshilflichen, stark auf potentiellen Diabetes verdächtigen Vorgeschichten wie übergewichtige Kinder und perinatales Absterben der Kinder. Der Test ist jedoch schwierig durchzuführen und zu interpretieren und häufig liegen die Ergebnisse im Grenzbereich. — Bei Personen mit starken klinischen Verdachtsmomenten in der Anamnese und pathologischem i.v.-G.T.T. war der orale G.T.T. häufig nicht in der Lage, die vermutete zugrundeliegende Abnormalität aufzudecken. — Der Cortison-G.T.T. zeigte sich empfindlicher als der orale und einfacher als der intravenöse G.T.T.; er scheint ein geeigneter Test für „Screening“-Zwecke in der Schwangerschaft zu sein. — Der Prednison-Glykosurie-Test zeigte in einem großen Teil falsch-positive Ergebnisse, besonders bei Personen mit renaler Glukosurie. Er ist deshalb in der Schwangerschaft von zweifelhaftem Wert. — Die Schwangerschaft scheint einen fortschreitenden diabetogenen Effekt zu haben, der bei prädisponierten Personen ausgeprägter ist; deshalb decken diese Tests Abnormalitäten im dritten Trimester mit größter Wahrscheinlichkeit auf. — Drei Frauen mit pathologischem i.v.-G.T.T. verloren ihre Kinder während der Schwangerschaft. Es wird angenommen, daß die Früherkennung des potentiellen Diabetes und die richtige geburtshilfliche Behandlung für die Senkung der perinatalen Mortalität von äußerster Wichtigkeit sind.

It is known that certain obstetrical complications and a high perinatal mortality may precede the onset of clinical diabetes by twenty or more years (MILLER, 1945, 1946; BARNES and MORGANS, 1948; PATON, 1948; MOSS and MULHOLLAND, 1951). It is also known that scrupulous antenatal care and better management of established diabetes in pregnancy can lower the perinatal losses in this condition. However, little has been achieved in this direction in pregnancies of women who have a potential risk of developing diabetes. This is due largely to the difficulties involved in the diagnosis of those potential diabetics (usually called "prediabetics") particularly in pregnancy.

A variety of tests of carbohydrate metabolism have been applied to pregnant women. Some have proved difficult to interpret and others have been used insufficiently to assess their worth. Indeed, there are no established tests that are agreed to be of value in detecting those with a potential risk of developing diabetes in pregnancy. Without some efficient tests it will be very difficult to make any great advance in the management of these pregnancies with their special hazards to both mother and child. For this reason, the present study was undertaken with the following aims:

1. To assess the carbohydrate tolerance in pregnant control women and in pregnant women suspected, on clinical grounds, of being potentially diabetic. To this end, four tests were used in combination: the oral glucose tolerance test (oral G.T.T.), the intravenous glucose tolerance test (intravenous G.T.T.), the cortisone-glucose tolerance test (cortisone-G.T.T.) and the prednisone-glycosuria test.

2. To evaluate the relative diagnostic value of these tests in the detection of potential diabetes in pregnancy by reference to clinical criteria suggesting this diagnosis.

3. To apply a suitable therapeutic regime for the reduction of perinatal mortality.

4. To initiate a long-term follow-up, which will be the final arbiter of the validity of any early deductions drawn from the results of this study.

It is recognized that no final decision can be reached on the ability of any laboratory tests to diagnose potential diabetes without adequate follow-up. It is known, however, from retrospective studies that certain clinical criteria, such as a high birthweight (more than 4.1 or 4.5 kg) and perinatal deaths, are associated with the later development of diabetes (SKIPPER, 1933; ALLEN, 1939; KRISS and FUTCHER, 1948; MOSS et al., 1951; PIRART, 1955). It therefore becomes possible to reach an interim decision on laboratory tests by comparing them with such clinical indications. This has been done to some extent in this study.

Subjects and methods

Subjects. 151 pregnant women were studied. All, except ten who were in-patients at the time of investigation, were attending the antenatal clinic of the Royal Maternity Hospital, Belfast. They were selected on the basis of a detailed clinical history, physical examination and urine analysis. They comprised two groups:

1. *Control group (A)*, of 50 women without any of the following: a family history of diabetes, glycosuria, a history of delivering an infant weighing more than 4.1 kg (9 lbs), obesity (weight over 91 kg or 200 lbs), abnormal obstetric history or any disorder known to affect carbohydrate metabolism. —

2. *Potential diabetes group (B)*, of 101 women presenting a history of one or more of the following:

a) Family history of diabetes in parents or siblings.
b) Past history of one or more babies weighing more than 4.1 kg.

c) Glycosuria (1 g per cent or more) on at least one occasion in the present or in previous pregnancies.

d) Abnormal obstetric history of stillbirth (s). However repeated abortions, a history of toxæmia, hydramnios and congenital malformations in infants were only regarded as possible indications of potential diabetes if associated with one or more of the criteria listed in a—f.

e) Obesity, defined as weight in excess of 91 kg at the time of the study.

Table 1. General characteristics and average time of testing for each group

Subject Characteristics	Group A (50)		Group B (101)	
	Mean	Range	Mean	Range
Age (years)	26.8	13—38	31.6	20—45
Height (cm)	163.3	150—167.6	161.0	145.0—175.0
Weight (kg)	68.7	52.0—89.7	75.2	48.0—97.8
Parity	1.82	1—7	4.0	1—14
Week of Testing:				
Second Trimester	19.0	11—26	19.0	10—26
Third Trimester	36.0	27—40	34.0	27—40

Table 2. The distribution of clinical indications in subjects of group B

No.	Clinical indications	Subjects
a	Family History { Parents	14
	Siblings	
b	Mothers of overweight babies	45
	{ 4.1 — 4.45 kg	20
	{ 4.5 kg or more	25
c	Glycosuria	42
d	Obstetric Complications	41
	Still-birth(s)	7
	All others	34
e	Obesity	22
f	Grand Multiparity	8

f) Grand multiparity, consisting of ten or more pregnancies. Criteria e and f were considered abnormal only when combined with at least one of the preceding criteria in a—d. The characteristics of both groups and the stage of pregnancy when the investigations were

carried out are shown in table 1. The distribution of clinical indications of group B is summarized in table 2.

Methods. The tests were performed from the tenth week onwards to full term and usually those done in the second trimester were repeated in the third. A standard diet of 250 g or more carbohydrate and around 2000 calories was prescribed for at least three days prior to each test. The three glucose tolerance tests were done in the morning after 12 to 15 hours' fast and all four were usually completed within two to four weeks. The steroid loading tests, that is cortisone-G.T.T. and prednisone-glycosuria test, did not precede the other two, unless sufficient time (one to two weeks) had been allowed for the steroid effect to pass off. Venous blood samples were used and the blood sugar estimation was done on the same day by the Hoffman (1937) method, modified for the "Technicon" Auto-analyser and yielding results which correspond to "true" blood sugar values.

Oral G.T.T. After drawing a fasting blood sample, the subject was given 50 g of glucose and subsequent blood specimens were taken at 1, 1½, 2, 2½ and 3 hours. For interpretation of the results, the criteria put forward by MOSENTHAL and BARRY (1950) were used. Thus, curves were interpreted as normal if peak and two-hour blood sugar values were less than 150 and 100 mg per cent respectively; and as abnormal when these limits were exceeded. In view of the larger amounts of glucose (100 g) used by these authors and the rather frequent occurrence of flat curves in pregnancy (LUND and WEESE, 1953; WEIDEN, 1948), these criteria were slightly modified to include as borderline, curves showing a two-hour value above 100 mg per cent with a peak lying between 120 and 150 mg per cent. Abnormal and borderline results were regarded as impaired or positive.

Intravenous G.T.T. The commonly employed technique was used (AMATUZZO et al., 1953; SILVERSTONE et al., 1957). With the subject lying in bed, a modified Lindeman needle was inserted into an antecubital vein and left *in situ* until the end of the procedure for the subsequent drawing of blood. After obtaining a fasting blood specimen, 50 ml of 50 per cent dextrose solution was given intravenously in the other arm within 2–4 minutes. Then, starting 8 minutes from the end of the injection, serial blood samples were collected at 8-minute intervals for 72 minutes (9 samples). The blood sugar values were then plotted on the ordinate of a semi-logarithmic graph paper against time on the abscissa; those falling between 16–24 and 56–64 minutes should form a straight line which, extrapolated to the interception with the ordinate, gives the theoretical blood sugar level at zero time. Thereafter, the rate of glucose utilization was calculated from the formula:

$$K = \frac{\log 2}{t_{1/2}} \times 100 \text{ or (since } \log 2 = 0.693) K = \frac{0.693}{t_{1/2}} \times 100$$

where, K the rate of utilization of glucose, expressed in mg per cent per min, and $t_{1/2}$ is the time, in minutes,

taken for the blood sugar concentration to reach half its value at zero time (as found by extrapolation of the straight line).

The value of K , thus obtained, is called the Total Index. Normal mean K -values for non-pregnant subjects were variously reported from 1.34 to 1.97 (HLAD and ELRICK, 1959). Because similar information for normal pregnancy is limited to that reported by SILVERSTONE et al. (1961, 1963), the criteria for normality used in this study are set forth in the section of "Results".

Cortisone-G.T.T. The procedure recommended by FAJANS and CONN (1959) was followed, except that a smaller dose of glucose (50 g) was used for loading. The result was considered abnormal if the blood sugar values at one and two hours were more than 160 and 140 mg per cent respectively; and borderline, if the one-hour value was more than 160 mg but the two-hour one lay between 130 and 140 mg per cent. This was done because loading doses of glucose less than 100 g tend to give a lower two-hour blood sugar value. (MOSENTHAL et al., 1950). Again, abnormal and borderline results were regarded as impaired or positive.

Prednisone-Glycosuria test. The original technique (JOPLIN et al., 1961) was used with minor modifications. The women were given an instruction sheet with details of the procedure which was also explained personally and subsequent questioning made sure whether the timing of urinary collection was properly observed; those failing to meet the requirements either had the test repeated or the result was excluded from the series. On the day of the test, 20 mg of prednisone was given orally at mid-day, 4 p.m. and 8 p.m. The subject fasted from 7 p.m. onwards. At 10 p.m. the bladder was emptied and from then until 6 a.m. inclusive, all urine was saved in a bottle containing chlorohexidine (Hibitane, 1:2000). The urinary sugar was estimated the same morning by the method used for blood. The non-sugar reducing substances were removed by first treating the urine with charcoal. The presence of other sugars, notably lactose, in urine was semi-quantitatively estimated by paper chromatography. The results of the test were expressed in mg of glucose excreted in urine per hour; in accordance with the original report, values up to 60 mg per hour were regarded as normal.

In a number of subjects, randomly selected from both groups, the intravenous G.T.T. and the prednisone-glycosuria test were repeated at various intervals during pregnancy and postnatally in order to study a) the reproducibility and b) the effect of pregnancy on the results.

Most of the tests and all biochemical estimations were done by one of us (A. B.).

Therapeutic plan. Subjects who, on grounds of the results, were considered potentially diabetic ("pre-diabetics") attended a special antenatal clinic. They were reviewed frequently and, in the third trimester, at weekly intervals. Obese women were put on a restricted carbohydrate diet (150–200 g) of 1500 to 2000

calories. Admission to the maternity hospital was arranged at 36 weeks and, whenever indicated on obstetrical grounds only, an early induction of labour by artificial rupture of membranes or a lower segment caesarean section was performed. Insulin, oral hypoglycemic agents or hormone therapy were not used.

Results

Intravenous G.T.T. Because published data on this test applying to pregnant women are inadequate to define the normal limits, the results obtained in the third trimester in our control series (group A) have been used to calculate these limits. Owing to the skew distribution of *K*-values in this group, a logarithmic transformation of the readings was carried out which resulted in an approximately normal distribution. From these transformed data it was found that the mean *K*-value was 1.73, the mean minus 1 S.D. lay at 1.40 and the mean minus 2 S.D. at 1.17. This is shown in Fig. 1.

Fig. 1. Intravenous G.T.T. Interpretation of results and their distribution in the third trimester

K-values between 1.39 and 1.18 were interpreted as borderline and those of 1.17 or less as abnormal. Borderline and abnormal *K*-values were regarded as suggestive of impaired (decreased) carbohydrate tolerance. (It must be stressed that these limits of normality refer only to the third trimester and that normal values earlier in pregnancy could not be calculated because of insufficient data.)

The effect of parity on *K*-values was studied in the control group and found to be not statistically significant at $P = 0.05$ (Table 3). The reproducibility of the test was assessed by repeating it in 21 subjects of both groups within the third trimester. The mean interval between tests was 5 weeks. The mean *K*-values on first and second testing were 1.45 and 1.46 respectively; the correlation coefficient (r) was 0.80, showing a high

Table 3. Mean *K*-values for control primigravidae subjects and those with one or two, and three or more pregnancies. Differences in parity are not statistically significant at $P = 0.05$

Parity	Subjects	Mean <i>K</i> -value	S.D.
Primigravidae	30	1.86	0.39
One or two pregnancies	10	1.68	0.23
Three or more pregnancies	10	1.68	0.22

degree of reproducibility (Fig. 2a). All 21 subjects investigated in the second trimester, had the test repeated in the third, within an average interval of 14 weeks. The mean *K*-values on first and second testing were 1.95 (S.D. = 0.33) and 1.61 (S.D. = 0.50) respectively and the correlation coefficient was 0.71 (Fig. 2b). The results of 58 tests done on group A and 135 tests on group B subjects are shown in Fig. 1 and in tables 4 and 5. Table 4 shows that *K*-values fall in both groups in later pregnancy. It also shows an obvious and significant difference between the groups, with group B having lower values. This lowering is also evident from table 5.

Table 4. Mean *K*-values and number of subjects studied in relation to period of pregnancy

	Group A		Group B	
	Before Third Trimester	During Third Trimester	Before Third Trimester	During Third Trimester
Number of Subjects	5	50	16	101
Number of tests	5	53 ¹	16	119 ¹
Mean Week (Range)	18.8 (12–26)	36 (27–40)	19.7 (10–26)	34.1 (27–40)
Mean <i>K</i> -value (S.D.) ²	2.51 (0.56)	1.73 (0.31)	1.78 (0.47)	1.51 (0.44)

¹ For tests performed twice on the same subject and in the same trimester, the arithmetic mean was taken for the analysis of results.

² Standard deviation.

Table 5. Intravenous G.T.T. The distribution of results in the third trimester

Result	Group A	Group B	Total
Normal (Mean – 1 S.D. ¹) $K \geq 1.40$	46	54	100
Borderline (Mean – 1 to – 2 S.D.) $K = 1.18$ to 1.39	4	27	31
Abnormal (Mean – 2 S.D.) $K < 1.17$	—	20	20
Total	50	101	151

¹ Standard deviation.

Fig. 2. Reproducibility of the intravenous G.T.T. and possible effect of the advancing pregnancy (2b)

In 16 women of group B, the test was repeated 2 to 16 weeks after delivery. The results, shown in table 6, demonstrate an overall rise in postnatal K-values.

Oral G.T.T. The results are shown in table 7 and their composite curve in Fig. 3. In the third trimester,

Table 6. Intravenous G.T.T. Comparative results of antenatal and postnatal tests (K-Values) obtained in group B

Name	Antenatal test		Postnatal test	
	Week	Result	Week	Result
S.Ev.	37	1.28	2	1.57
D.R.	38	1.03	2	1.36
A.P.	37	1.16	2	0.87
C.M.	37	1.28	2	1.17
B.M.	37	1.16	2	1.26
M.V.	35	1.00	6	1.02
S.E.	38	1.28	6	1.03
R.E.	38	1.31	7	1.55
S.J.	37	1.02	13	1.12
T.A.	36	0.80	13	0.78
M.D.	28	0.94	13	1.05
N.C.	34	0.83	6	1.38
S.M.	27	0.98	10	1.90
H.H.	38	1.46	5	3.15
V.A.	38	1.41	5	2.23
H.E.	39	1.41	16	2.77
Mean	35.9	1.15	6.9	1.50

Table 7. Results of oral and cortisone-G.T.T.

Result	Oral G.T.T.			Cortisone G.T.T.		
	Group A	Group B	Total	Group A	Group B	Total
Second Trimester						
Normal	5	20	25	3	2	5
Borderline	—	3	3	—	1	1
Abnormal	—	5	5	—	1	1
Total	5	28	33	3	4	7
Third Trimester						
Normal	44	71	115	31	33	64
Borderline	4	8	12	2	5	7
Abnormal	2	8	10	2	7	9
Total	50	87	137	35	45	80

there were 4 positive results among the 50 subjects of group A (12%) and 16 similar results among 87 subjects of group B (18.4%) tested in this period.

Statistical analysis of results showed no significant difference between groups A and B. A highly significant difference ($P = 0.005$), however, was found on comparing the results between subjects with normal and impaired intravenous G.T.T.

Cortisone-G.T.T. The results are shown in table 7 and their composite curve in Fig. 4. Positive results in the third trimester were seen in 4 out of 35 subjects of group A (11, 4%) and in 12 out of 45 subjects of group B (26.6%). As with the previous test, comparison of results between the groups did not reveal any significant difference. ($P > 0.05$). —

Fig. 3. Oral G.T.T. Composite curves for the second and third trimesters

Prednisone-glycosuria test. The results are shown in table 8. It will be seen that 13 out of 42 women in group A (31%) and 61 out of 92 women in group B

(66.3%) showed abnormal tests in the third trimester, a number significantly larger than in the previous tests.

In Fig. 5 the results are compared with those of the intravenous G.T.T. This shows that in patients within group B there was no agreement between the two tests. It also suggests that, in the majority of cases, the glycosuria was of renal origin. The reproducibility of the test was assessed by repeating it in 18 subjects of both groups within a 5-week mean period in third

Fig. 4. Cortisone G.T.T. Composite curves for the third trimester

Table 8. Results of prednisone-glycosuria test

Result	Group A	Group B	Total
Second Trimester			
Normal	3	11	14
Abnormal	2	5	7
Total	5	16	21
Third Trimester			
Normal	29	31	60
Abnormal	13	61	74
Total	42 ¹	92 ¹	134

¹ For tests done more than once on the same subject, the arithmetic mean of the results was taken for interpretation.

trimester. The correlation coefficient was 0.79. In contrast, 19 tests done in early pregnancy and repeated in the third trimester, within a mean period of 14.5 weeks, showed a poor correlation coefficient, 0.19 (Fig. 6a and 6b).

In 19 women, randomly selected, the test was repeated 2 to 16 weeks after delivery. As shown in table 9, a considerable reduction of glycosuria with reversion of most abnormal results to normal was noted in postnatal period. Paper chromatography of urine in 20 specimens showed the presence of lactose in only 7 and then in low concentrations (2–15 mg per cent), suggesting that lactosuria was not responsible for the abnormal results (Table 9).

The outcome of pregnancy.

Group A. Fifty live infants were delivered to 50 women; forty five weighed less than 4.1 kg and a further five less than 4.5 kg (10 lbs) at birth. Caesarean section was performed in five cases; there was no maternal death.

Fig. 5. Distribution of results of the prednisone-glycosuria test. In group B the results are compared with those obtained by the intravenous G.T.T.

Fig. 6. Reproducibility of the prednisone-glycosuria test and possible effect of the advancing pregnancy (6b)

Table 9. *Prednisone-glucosuria test. Comparative results of antenatal and postnatal tests*

No.	Name	Antenatal test		Postnatal test	
		Week	Result	Week	Result
1.	H.S.	34	66	2	18
2.	D.R.	38	400	2	62
3.	A.P.	36	130	2	28
4.	GLM.	37	819	2	141
5.	B.M.	37	112	2	32
6.	M.V.	35	608	6	32
7.	Sh.E.	38	196	9	140
8.	R.P.	35	445	1	34
9.	S.J.	37	48	13	27
10.	T.A.	35	175	13	31
11.	M.D.	28	3.050	12	1.636
12.	N.C.	36	247	6	15
13.	S.M.	27	37	11	20
14.	Tu.M.	39	5.106	2	994
15.	V.A.	39	407	4	38
16.	Ho.E.	39	60	16	28
17.	Hac. E.	38	342	1	121
18.	Ho J.	33	363	4	186
19.	W.J.	40	78	3	97

Group B. In all 99 live infants, including a pair of twins, were delivered. There were three perinatal deaths (two stillbirths, one death ten hours after birth). Seventy-six infants weighed less than 4.1 kg, nineteen between 4.1 and 4.45 kg, and seven 4.5 kg or more. Thirteen caesarean sections were performed and there was no maternal death.

Relationship between clinical indications and results of tests.

Family history. Fourteen subjects had a close family history of diabetes (12 one parent or sibling, 2 both parents); nine of them, including the last two, had normal oral and intravenous GGTs.

Overweight infants. Regarding past pregnancies it was found that, in the group of 25 mothers with infant-birthweight 4.5 kg or more, 21 had impaired intravenous G.T.T.s (K -values less than 1.40) and 7 had borderline or abnormal oral G.T.T.s. Similar results were also obtained in 6 out of 12 women who had the cortisone G.T.T. On the other hand, impaired intravenous G.T.T.s were seen in only 5 out of 20 mothers with infant-birthweight 4.1 to 4.45 kg. The difference was highly significant ($P < 0.001$).

Similar observations were made in the present pregnancy; six out of seven infants weighing 4.5 kg or more were born to mothers with impaired intravenous G.T.T.s.

Glycosuria. Twenty-one out of 42 subjects referred for glycosuria had impaired intravenous G.T.T.s.

Perinatal loss. Six out of seven women with a past history of stillbirths and all three who lost their babies in the present pregnancy had impaired intravenous G.T.T.s. In contrast, oral testing was positive in only two and one instances, respectively.

Obesity and parity. Among the 22 obese women there were 15 who had impaired intravenous G.T.T.s. and 6 who had positive oral G.T.T.s. The mean parity in obese subjects was 4.6 against 3.9 in non-obese ones.

The numbers studied were too few, however, to determine the effect of obesity and parity separately.

Discussion

There was a difference in mean age, weight and parity between the groups which could hardly be avoided, since obesity and multiparity were included in the criteria used for the selection of group B subjects. Regarding age and weight, it is known that differences of this range do not affect the results of the tests (SILVERSTONE et al., 1957; GERMAN, 1958). Parity, on the other hand, is postulated to have a lowering effect on carbohydrate tolerance (PYKE, 1956; FITZGERALD et al., 1961) but the difference in our study was not proved to be statistically significant.

Comparative assessment of the results confirms the view that none of the currently available tests seems to be adequately reliable for the investigation of carbohydrate tolerance in pregnancy. It helps however to clarify the relative merits of each test. This is mainly true for the *intravenous G.T.T.*, which has been reported to give inconclusive or controversial results in normal and potentially diabetic pregnancy. Thus while early reports, reviewed by BURT (1960), were consistent with his observations in showing no differences between normal pregnant and non-pregnant women, SILVERSTONE et al. (1961) found a considerable increase of glucose utilization in pregnancy. Our results are in keeping with the latter observations but only for tests done before the third trimester, when high K -values were found in all subjects of group A and in those of group B who were subsequently shown to have no impairment of carbohydrate tolerance.

In potentially diabetic ("prediabetic") pregnancy — on grounds of clinical history and abnormal oral G.T.T.s — KAPLAN (1961) found the intravenous G.T.T. normal, but WELSH (1960) reported abnormal K -values in 30 per cent of women. Our results lend support to Welsh's findings; as shown earlier, low K -values were found in most women with history of overweight infants and/or stillbirths in past pregnancies as well as in the present one.

There were four borderline results in the control group, yielding an 8 per cent incidence of "false positive" responses. However, this is not unduly high considering that, according to the statistical assumptions of the classification of results, 13 per cent of all responses from either group could be borderline (between one and two S.D.) and another 3 per cent abnormal (less than two S.D.). Three of those positive responses occurred in primigravidae control subjects who had also impaired oral and cortisone-G.T.T.s. It is not unlikely, therefore, that they represented mild and asymptomatic cases of toxæmia, which is known to decrease the carbohydrate tolerance (BURT, 1955).

Reproducibility was satisfactory only for tests repeated within a short interval; when this was longer (3–5 months), lower values were usually obtained on second testing in subjects of either group. It is therefore

suggested that a gradual decrease in carbohydrate tolerance occurs as pregnancy advances, most manifest in the third trimester. This is consistent with the experimental evidence of insulin resistance in late pregnancy (BURT, 1958) and the progressive increase in the number of abnormal glucose tolerance tests seen in potentially diabetic subjects during pregnancy (PALMER et al., 1957; CARRINGTON et al., 1958).

Possible limitations of the test are the intricate procedure and calculations involved and a relatively high proportion of borderline results, which are likely to include a fair number of false positive responses. Summing up our experience with this test, we think that it offers a useful diagnostic tool for investigation of potentially diabetic pregnancy.

The Oral G.T.T. was found less informative in comparison with the intravenous test. Although 88 per cent of the positive responses in group B were found in subjects with impaired intravenous tolerance, it is noteworthy that the test was normal in most women with obstetric history suggestive of potential diabetes (large infants and stillbirths) and impaired intravenous G.T.T.s. Our impression is that, as JACKSON and WOOLF (1958) point out, it fails not infrequently to disclose an underlying abnormality of carbohydrate tolerance.

The cortisone-G.T.T. was reported to yield a considerable number of false positive and false negative results in potentially diabetic pregnancy (JACKSON, 1961). However, in this study most subjects of group B with positive responses had also impaired intravenous G.T.T.s (10 out of 12) and a strong history of potential diabetes, including six women with a past history of babies weighing 4.5 kg or more. The test is simpler to perform than the intravenous and appears more sensitive than the oral one; it is felt, therefore, that it forms a suitable technique for screening purposes in pregnancy.

Oral and cortisone-G.T.T. showed four positive results each in the control group. As noted earlier though, three of them occurred in the same subjects who had, in addition, impaired intravenous G.T.T.s, suggesting that this apparently high incidence might be due to their inappropriate inclusion as controls.

This study failed to confirm the original claims for the diagnostic merits of the prednisone-glycosuria test. JOPLIN et al. (1961) observed no abnormal responses in their control pregnant women as compared to 31 per cent in our series. The test did not show diagnostic specificity in relation to infant birthweights and, in the presence of glycosuria, abnormal results can be expected in 90 per cent of subjects. It would thus appear, that it is not of much help for investigation of pregnancy. The postnatal return to normality of most abnormal results was possibly due to the abolition of some gestational effect on renal dynamics (WELSH and SIMS, 1960).

Two minor differences from the originally proposed technique, which might cause criticism, warrant a brief comment. First, the test was carried out mostly

on an out-patient basis, as it was felt scarcely justifiable to admit apparently healthy women for investigations. It is possible that, despite the detailed instructions and exclusion of suspicious results, a few inaccurate tests might still have been included in the series. Second, the use of a non-specific method for urinary glucose estimation might have resulted in high figures. The chromatography of urine, however, showed the presence of lactose in only one-third of specimens and then in such small quantities (2–15 mg per cent), as to be unlikely to affect the results.

Acknowledgements. We are most grateful to Dr. D.A.D. MONTGOMERY, Physician-in-charge of the Metabolic Unit, for his valuable guidance and constant encouragement throughout this study.

We also express our thanks to: Professor G.M. BULL, for helpful suggestions and constructive criticisms; Professor C.H. MACAFEE and Dr. J.M.G. HARLEY for their advice on the obstetrical aspects of the work and for referring most of the subjects; Messrs G. BOYD, G.B. GIBSON, H.I. MCCLURE, and R.A.E. MAGEE, for kind permission to study patients under their care; Professor E. A. CHEESEMAN and Mr. E. TURKINGTON for statistical advice; and Mr. D. NEILL, for laboratory facilities.

One of us (A.B.) gratefully acknowledges the financial support received from the Northern Ireland Hospitals Authority through a Research Fellowship.

References

- ALLEN, E.: The glycosurias of pregnancy. *Amer. J. Obstet. Gynec.* **38**, 982–992 (1939).
- AMATUZIO, D.S., F.L. STUTZMAN, M.J. VANDERBILT and S. NESBITT: Interpretation of the rapid intravenous glucose tolerance test in normal individuals and in mild diabetes mellitus. *J. clin. Invest.* **32**, 428–435 (1953).
- BARNES, H.H. F., and M.E. MORGANS: Prediabetic pregnancy. *J. Obstet. Gynaec. Brit. Emp.* **55**, 449–454 (1948).
- BURT, R.L.: Peripheral utilization of glucose in pregnancy. II. Preeclampsia. *Obstet. and Gynec.* **6**, 51–54 (1955).
- Reactivity to tolbutamide in normal pregnancy. *Obstet. and Gynec.* **12**, 447–453 (1958).
- Carbohydrate metabolism in pregnancy. *Clin. obstet. gynec.* **3**, 310–315 (1960).
- CARRINGTON, E.R., H.S. REARDON and C.R. SHUMAN: Recognition and management of problems associated with prediabetes during pregnancy. *J. amer. med. Ass.* **166**, 245–249 (1958).
- FAJANS, S.S., and J.W. CONN: The early recognition of diabetes mellitus. *Ann. N.Y. Acad. Sci.* **82**, 208–218 (1959).
- FITZGERALD, M.G., J.M. MALINS and D.J. O'SULLIVAN: Prevalence of diabetes in women thirteen years after bearing a big baby. *Lancet* **1961** **I**, 1250–1252.
- GERMAN, J.L.: The glucose tolerance test after cortisone administration in obese and nonobese men. *Diabetes* **7**, 261–266 (1958).
- HLAD, C.L., Jr., and H. ELRICK: Further studies of the kinetics of glucose utilization. I. A new method of data analysis. *J. clin. Endocr.* **19**, 1258–1273 (1959).
- HOFFMAN, W.S.: Rapid photoelectric method for determination of glucose in blood and urine. *J. biol. Chem.* **120**, 51–55 (1937).
- JACKSON, W.P.U., and N. WOOLF: Maternal prediabetes as a cause of the unexplained stillbirth. *Diabetes* **7**, 446–448 (1958).

- The cortisone-glucose tolerance test with special reference to the prediction of diabetes. Diagnosis of prediabetes. *Diabetes* **10**, 33–40 (1961).
- JOPLIN, G.F., R. FRASER and K.J. KEELEY: Prednisone-glycosuria test for prediabetes. *Lancet* **1961 II**, 67–71.
- KAPLAN, N.M.: Tolbutamide tolerance test in carbohydrate metabolism evaluation. *Ann. Int. Med.* **107**, 212–224 (1961).
- KRISS, J.P., and P.H. FUTCHER: The relation between infant birthweight and subsequent development of maternal diabetes mellitus. *J. clin. Endocr.* **8**, 380–389 (1948).
- LUND, C.J., and W.H. WEESE: Glucose tolerance and excessively large babies in nondiabetic mothers. *Amer. J. Obstet. Gynec.* **65**, 815–829 (1953).
- MILLER, H.C.: The effect of the prediabetic state on the survival of the fetus and the birth weight of the newborn infant. *New Engl. J. Med.* **233**, 376–378 (1945).
- The effect of diabetic and prediabetic pregnancies on the fetus and newborn infant. *J. Pediat.* **29**, 455–461 (1946).
- MOSENTHAL, H.O., and E. BARRY: Criteria for and interpretation of normal glucose tolerance tests. *Ann. Int. Med.* **33**, 1175–1194 (1950).
- MOSS, J.M., and H.B. MULHOLLAND: Diabetes and pregnancy with special reference to the prediabetic state. *Ann. Int. Med.* **34**, 678–691 (1951).
- PALMER, L.J., W.P. GIVEN, E.L. SLEVIN, P. WHITE and H. WILKERSON: Pregnancy and diabetes. A panel discussion. *Diabetes* **6**, 523–533 (1957).
- PATON, D.M.: Pregnancy in prediabetic patient. *Amer. J. Obstet. Gynec.* **56**, 558–560 (1948).
- PIRART, J.: The so-called prediabetic syndrome of pregnancy. *Acta endocr. (Kbh.)* **20**, 192–208 (1955).
- PYKE, D.A.: Parity and the incidence of diabetes. *Lancet* **1956 I**, 818–820.
- SILVERSTONE, F.A., M. BRANDFONBRENER, N.W. SHOCK and M.J. YIENGST: Age differences in the intravenous glucose tolerance tests and the response to insulin. *J. clin. Invest.* **36**, 504–514 (1957).
- E. SOLOMONS, and J. RUBRICIUS: The rapid intravenous glucose tolerance test in pregnancy. *J. clin. Invest.* **40**, 2180–2189 (1961).
- — — The rapid intravenous glucose tolerance test in obstetrical patients with a family history of diabetes. *Diabetes* **12**, 398–405 (1963).
- SKIPPER, E.: Diabetes mellitus and pregnancy. A clinical and analytical study. *Quart. J. Med.* **2**, 353–380 (1933).
- WEIDEN, S.: Investigation of carbohydrate metabolism in normal pregnancy. *Med. J. Aust.* **I**, 646–652 (1948).
- WELSH, G.W., 3rd, and E.A.H. SIMS: The mechanisms of renal glucosuria in pregnancy. *Diabetes* **9**, 363–369 (1960).
- Studies of abnormal glucose metabolism in pregnancy. *Diabetes* **9**, 466–470 (1960).

A. BILLIS
Department of Medicine
King Paul's Hospital
Athens/Greece