THE INFLUENCE OF NORMAL PREGNANCY ON CARBOHYDRATE TOLERANCE.

by

David J. Mackay,

M.B., Ch.B., M.R.C.O.G.

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PREFACE.

The investigations on which this thesis is based were carried out in the Research Department and wards of the Glasgow Royal Maternity Hospital, and in the wards of the Royal Samaritan Hospital for Women, Glasgow.

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INTRODUCTION.

While traditionally, it has been taught that pregnancy alters glucose tolerance there are wide differences of opinion on this subject. Authors such as Johnson and Bosnes (1948), Jackson (1952) and Marazzini (1957) claim that carbohydrate tolerance is not impaired in normal pregnant women; others, notably Hoet (1954 ; 1957) go so far as to state that pregnancy is actually diabetogenic.

Resolution of this controversy is a matter of considerable practical importance because it may have a bearing not only on the obstetric management of the established diabetic, but also in the recognition of the prediabetic stage of the disease. One factor which may present difficulties is the tendency for glycosuria to occur in a number of pregnancy states.

When glycosuria is found in association with normal blood sugar values, it is customarily ascribed to lowering of the renal threshold as conceived by Claude Bernard and assumed to be physiological. The inference is commonly made that the urinary sugar in these cases has been

detected as a matter of chance (i.e. post-prandially) and that the carbohydrate metabolism of such cases conforms to that found in normal pregnancy. It is obvious from the views quoted that this is not universally accepted. It might well be that glycosuria is a manifestation of some intrinsic defect peculiar to these women. Consequently elucidation of this matter has been one of the aims of these studies.

In addition to diabetes and prediabetes, there are a number of frankly abnormal conditions in pregnancy where the finding of urinary sugar is a common clinical experience. These include haemorrhagic shock, hyperemesis gravidarum and prolonged labour with ketosis.

The excretion of sugar in association with shock resulting from haemorrhagic conditions such as accidental haemorrhage, post-partum haemorrhage, abortion and uterine rupture is now understandable. It has been demonstrated that blood sugar levels are frequently abnormally high where such conditions obtain (Murdoch, 1953). Moreover, findings comparable to these have been reported in

situations of stress occasioned by severe burn injuries and acute specific infections in the non-gravid (Lisle and Dick, 1932).

However, the mechanism by which sugar is excreted in prolonged labour and severe hyperemesis gravidarum is clearly quite different. In these circumstances the associated ketonuria is an indication of depleted carbohydrate reserves and there is no hyperglycaemia. The urinary loss of sugar is therefore paradoxical in such cases.

In recent years obstetricians have become increasingly impressed with the importance of prediabetes as a clinical entity. Aspects of the complete syndrome of large babies, a high rate of foetal loss, the tendency to hydramnios and toxaemia of pregnancy, as described by Allen (1939) have subsequently been remarked by many others including Miller et al., (1944) ; Kriss and Futcher (1948) and Gilbert and Dunlop (1949).

Stowers (1961), in discussing the hazards of delay in the recognition and treatment of diabetes, stresses the urgency of diagnosis in

the pre-diabetic stage by means of sensitive tests for carbohydrate intolerance. Following the demonstration by Ingle (1941a.), that cortisone induces hyperglycaemia and glycosuria when given in large and sufficiently prolonged dosage to experimental animals, Berger (1952) suggested the administration of cortisone as the basis of such provocative tests. As a result, several authors including Fajans and Conn (1954) and Duncan (1956b.) have claimed considerable success in the non-gravid. More recently Bertrand and Russell (1960) have reported rather equivocal results using prednisone in parous women.

Such tests have been disappointing in unmasking the prediabetic state during pregnancy. There are two possible explanations for this. It has been shown that there is an increase in corticosteroid secretion as pregnancy advances (Parviainen et al., 1950 ; Bayliss et al., 1955 ; Schuller 1957 ; Cassano and Tarantino 1957), so that the doses empirically employed may be relatively ineffectual. Again there is, as already indicated, confusion and dispute as to

what constitutes normal behaviour with regard to carbohydrate tolerance in pregnancy, and this may well be the more important. As a result, the diagnosis of prediabetes in the gravid patient remains largely speculative, being based on the obstetric history rather than on more precise scientific analysis.

According to Peel (1959) the management of pregnancy in the established diabetic is becoming increasingly important because of the increasing frequency with which the two conditions are associated. Indeed it would appear that the problem of fecundity has now replaced that of infertility. As Joslin (1959) points out, this problem is likely to increase owing to the transmission of a hereditary gene.

While it is true that the maternal mortality from diabetes mellitus has fallen dramatically to 1.4 per cent with insulin therapy, (Peel and Oakley 1949 ; Oakley, (1953) the disease is still associated with a high foetal wastage in the last eight weeks of gestation and in early neonatal life. With the exception of the unique salvage

rate of 90 per cent achieved by White (1947 : 1959) using oestrogen and progesterone therapy, which will be considered in greater detail later, the foetal wastage ranges from 15 to 41 per cent (Oakley 1953 ; Peel (1955 ; 1959) ; Pedersen and Brandstrup 1956). According to these authors, this depends on the degree of combined medical and obstetrical care.

It is common experience that the gravid diabetic may be extremely difficult to stabilise, particularly in the last trimester, and that even in apparently well stabilised cases there is no guarantee of a successful outcome as far as the child is concerned. As a result, premature delivery at 36 to 38 weeks is the generally accepted policy. This incurs a considerable risk of neonatal loss from immaturity.

While it is agreed that changing metabolic requirements inherent in the diabetic state may be partly responsible for unsuccessful stabilisation, it is possible that normal physiological changes in carbohydrate utilisation

and excretion may also be concerned. In this connection it is well recognised that urinary sugar estimations are an unreliable guide to stabilisation in pregnancy, but it has never been altogether clear as to how far this is due to normal physiological metabolic changes. For example, the presence of lactose in the urine might result in an erroneous appreciation of the patients' insulin requirements.

It would appear from the literature that the incidence of lactose in pregnancy urine is a subject about which there is some uncertainty. A minority of writers, including Selye (1947), claim that lactosuria is common during lactation but does not occur during pregnancy. The majority, however, agree with Lambie (1926) that lactose may, on occasion, be excreted in very late pregnancy in addition to the puerperium. Since it seemed possible that both of these views might be too conservative, the incidence of lactosuria in relation to the stage of gestation has been investigated, in these studies.

The introduction and wide adoption of glucose-specific test strips have to no degree reduced the clinical importance of this problem. These oxidase tests are extremely sensitive and essentially qualitative. Consequently where positive results have been obtained, the concentrations of sugar must be checked by one of the accepted non-specific clinical methods (Jablokow et al., 1957). Hence, should glucose and lactose occur together, the latter sugar might give an exaggerated impression of the concentration of glucose actually present in the specimen. Furthermore, it seemed equally possible that other sugars might contribute to reductions in the urine of pregnant women in a similar fashion.

In view of the divisions of opinion and the practical importance of establishing the influence of normal pregnancy on carbohydrate tolerance and insulin utilisation, a series of studies has been conducted on normal gravid and non-gravid women. Throughout this series paper chromatography has been employed for the positive identification of the individual sugars excreted in the urine.

For the sake of clarity the work is divided into three main sections:

I Preliminary Study.

II The Assessment of Carbohydrate Tolerance by Means of Intravenous "Loads".

III The Assessment of Carbohydrate Tolerance by Means of Continuous Infusion.

Thereafter the results are discussed and conclusions drawn.

SECTION I. PRELIMINARY STUDY.

THE INFLUENCE OF AGE, PARITY AND THE STAGE OF GESTATION ON URINARY SUGAR OUTPUT, AND THE TYPES OF SUGAR EXCRETED IN NORMAL PREGNANCY.

As a preliminary study it was decided to investigate the frequency with which clinically detectable amounts of glucose, lactose, and any other reducing sugars are present in normal pregnancy urine, and to establish whether their excretion could be related to age, parity or to any particular stage of gestation.

MATERIAL AND METHODS.

A sample of 21 primigravidae and 20 multiparous women was obtained in consecutive order of their first attendance at the ante-natal clinic. Only those having pregnancies of less than 21 weeks duration were included. Of the initial 41 cases, 2 were found subsequently to be non-gravid. This left 39 cases consisting of 21 primigravidae and 18 parous women. Of these, 1 aborted at 16 weeks, 1 was delivered

of an anencephalic foetus at 30 weeks and 1 was delivered of a premature but otherwise healthy infant in the 36th week of gestation. Two of the cases had their latter ante-natal care and confinement elsewhere.

Thus, for purposes of analysis, the final series consisted of 34 cases of whom 18 were primigravidae and 16 were parous.

At their initial and all subsequent attendances a specimen of urine was obtained. This usually was the first to be passed after lunch.

The specimen was first tested for sugar by two methods, viz. "Clinitest" and Benedict's qualitative solution in order that the investigation would concern itself with only such quantities of reducing substances as were detectable by clinical methods.

As a means of differentiating between reductions caused by glucose and lactose, fermentation tests and the preparation of

osazones have been recommended. (Harrison 1947 ; Hawk et al., 1947 ; Varley 1954). These methods have not in fact proved suitable in obstetric practice. Fermentation is too elaborate and time-consuming for routine work, particularly when a large number of specimens have to be examined. Furthermore, the concurrent presence of glucose and lactose may render the osazone test of doubtful value. Another important objection to these tests was the possibility that sugars other than glucose or lactose - whether from a dietary or metabolic source - might be present either individually or severally. In view of these considerations, it was decided to examine all specimens of urine by the more informative and sensitive method of paper chromatography, where one or other of the more conventional clinical methods had yielded a positive reduction.

For this purpose the technique described by Williams (1954) was eventually selected as being the most suitable of the methods tried

(Appendix A). A positive result was accepted only where the presence of sugar was confirmed by chromatography. When a sugar other than glucose, lactose, or galactose was detected, a second chromatogram was run for confirmation.

In general, the patients reported at monthly intervals until the 28th week of gestation, fortnightly until the 32nd. week, and then at weekly intervals until delivery. This accounts for the increased frequency of testing in late pregnancy.

In all, 288 specimens of urine were thus examined.

RESULTS.

The results are shown in Tables A (Appendix B) and I.

False positive reductions were obtained in 37 (12.8 per cent) of the tests. All were obtained by Benedict's qualitative method and were for trace quantities (i.e. 25 mg. per cent or less). In every case where "Clinitest" registered a reduction, the

TABLE I.

	Weeks of Gestation.			
	Under 21	21 - 30	31 - 35	36 to term
Number of tests on 34 patients	52	75	71	90
Negative tests (including False Positives).	42 (6) 80.8%	41 (13) 54.7%	26 (9) 36.6%	37 (9) 41.1%
Positive Reductions.	10 19.2%	34 45.3%	45 63.4%	53 58.9%
Reductions caused by:-				
Glucose	4	9	6	4
Lactose	2	13	18	27
Glucose + Lactose	2	9	13	13
Glucose + Lactose + Galactose	1	1	2	3
Glucose + Lactose + Pentose	-	-	1	1
Lactose + Galactose + Pentose	-	-	1	-
Fructose + Galactose	-	1	-	-
Pentose	1	-	-	-
Fructose + Galactose + Lactose	-	1	1	2
Glucose + Galactose	-	-	2	-
Galactose	-	-	1	1
Glucose + Fructose	-	-	-	1
Lactose + Galactose	-	-	-	1
Number of Patients with Urinary Glucose	7 20.6%	14 41.0%	17 50%	13 38.2%
Lactose	5 14.7%	16 47.0%	18 52.9%	26 76.5%
Galactose	1 2.9%	2 5.9%	5 14.7%	6 17.6%
Pentose	1 2.9%	- 5.9%	2 5.9%	1 2.9%
Fructose	- -	1 2.9%	1 2.9%	3 8.8%

presence of carbohydrate was confirmed by chromatography.

The earliest positive reduction was obtained at 16 weeks. This was of the order of 25 mg. per cent and was caused by glucose and lactose concurrently. The maximum concentration of sugar recorded in the series was 100 mg. per cent. The sugar was identified as glucose. The concentration of lactose did not exceed 50 mg. per cent on any occasion.

Only one of the series of 34 women completed gestation without having sugar detected in her urine. This pregnancy was concluded normally.

The results of the tests for individual patients are given serially under the corresponding week of gestation in Table A (Appendix B).

For purposes of analysis, these results have been grouped into the four periods viz., up to 21 weeks, 21 to 30 weeks, 31 to 35 weeks and 36 weeks to term (Table I).

The number of tests in each period is shown

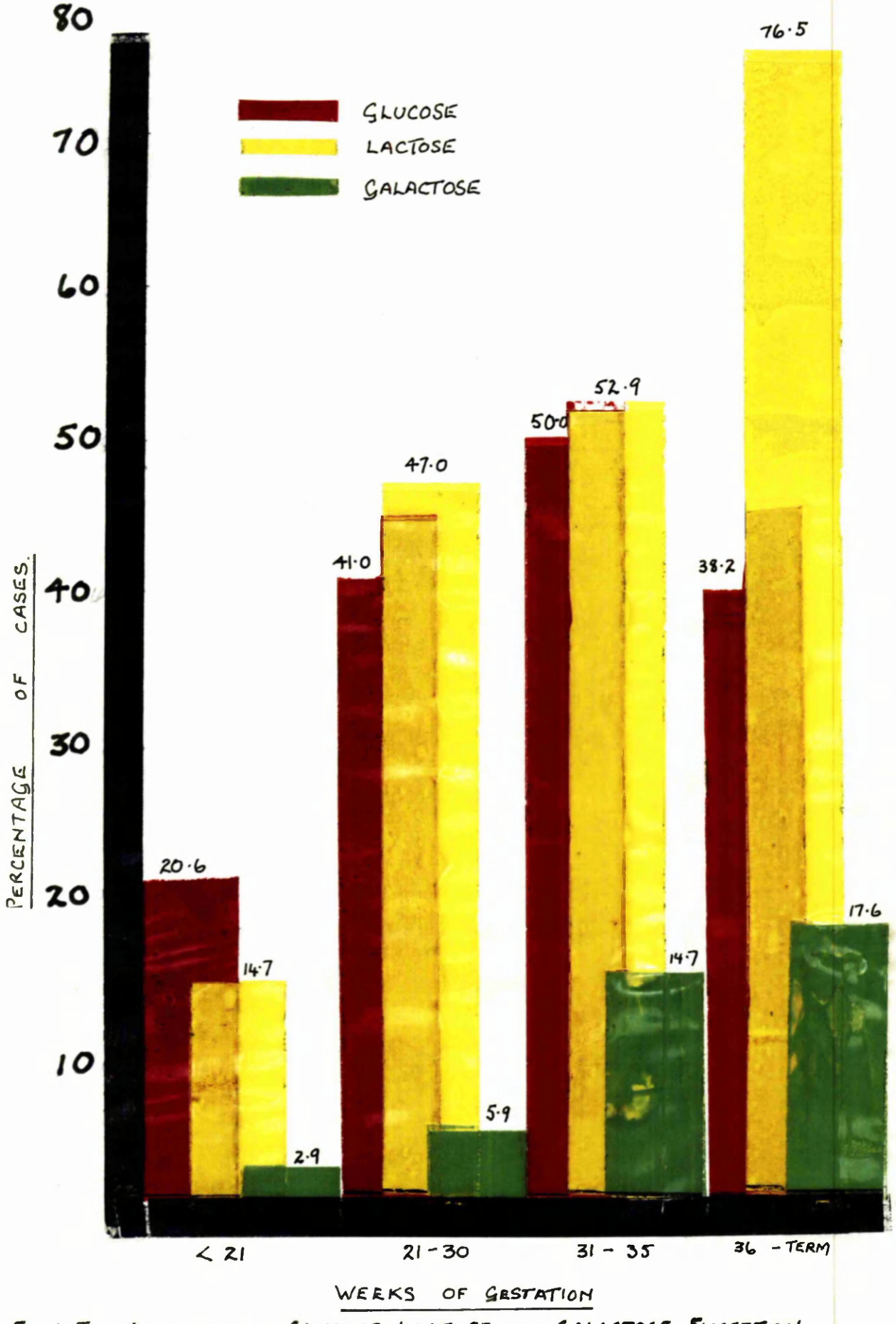


FIG. 1 THE INCIDENCE OF GLUCOSE, LACTOSE AND GALACTOSE EXCRETION IN RELATION TO THE STAGE OF GESTATION.

and the results are tabulated according to their being negative (including false positives, shown in brackets) or positive reductions. The positive reductions in each period are further detailed according to the sugar(s) contributing. Finally, in the same table, is the number of patients having a particular sugar in their urine at any of the four periods.

Lactose, glucose and galactose were the sugars most commonly detected, and Figure I illustrates the percentage of cases in whose urine these were present as normal pregnancy advanced.

It will be observed that there was a progressive increase in the incidence of lactosuria, from early pregnancy until term. The same appears to be true of galactose. The trend with regard to glucose excretion is less clear. The apparent maximum incidence in the 31 - 35 weeks interval, and the fall in the 36 weeks to term period, are not supported by statistical analysis.

With regard to the Presence (+) or

TABLE 2.

PRESENCE (+) or ABSENCE (-) of GLUCOSE.

Case No.	Up to 21 weeks	21-30 weeks	31-35 weeks	36 weeks to term
1.	-	-	+	+
2.	-	+	-	-
3.	-	-	-	-
4.	-	+	+	-
5.	-	-	+	-
6.	-	-	-	-
7.	-	+	+	+
8.	+	+	+	-
9.	-	-	+	+
10.	+	+	+	+
11.	-	-	-	+
12.	-	-	+	-
13.	-	+	+	-
14.	+	+	+	+
15.	+	-	-	-
16.	-	-	-	-
17.	-	+	+	+
18.	-	-	-	-
19.	-	-	+	-
20.	+	+	-	+
21.	-	+	+	-
22.	-	+	-	-
23.	-	-	-	-
24.	-	-	-	-
25.	-	+	-	+
26.	-	-	-	-
27.	+	-	+	+
28.	-	-	+	+
29.	-	-	-	-
30.	-	-	+	+
31.	-	-	-	-
32.	-	+	-	-
33.	+	+	+	+
34.	-	-	-	-

Absence (-) of Glucose (Table 2), the various periods may be considered as follows:-

(1) Table 3.

21-30 weeks	Up to 21 weeks +	Up to 21 weeks -	Total
+	5	9	14
-	2	18	20
Total	7	27	34

It will be noticed that 5 cases were ++ (the first sign refers to the "up to 21 weeks" period) and 18 were --. Thus 23 cases showed no change. Of the remaining 11 one would expect, if only chance operated, $\frac{11}{2}$ = 5.5 in the -+ and $\frac{11}{2}$ = 5.5 in the + - group. We actually observe 9 and 2. An X^2 test shows that these observed frequencies are significantly different from 5.5 and 5.5. (X^2 = 4.9, $P<0.05$). Thus the proportion of +ve's, 7 out of 34, in the "up to 21 weeks" is significantly different from the proportion of +ve's, 14 out of 34, in the 21 - 30 weeks period.

(11) Table 4.

31-35 weeks.	21-30 weeks. +	21-30 weeks. -	Total.
+	9	8	17
-	5	12	17
Total	14	20	34

An X^2 test shows that there is no significant difference between the proportion of +ve's in the two periods. That is, there is no significant difference between $\frac{14}{34}$ and $\frac{17}{34}$.

(111) Table 5.

36 weeks to term.	31-35 weeks +	31-35 weeks -	Total
+	10	3	13
-	7	14	21
Total	17	17	34

Again there is no significant difference between proportions of +ve's. That is to say there is no significant difference between $\frac{17}{34}$ and $\frac{13}{34}$. In short, whereas there is a

significant increase from 21 to 30 weeks, over the period under 21 weeks ($P<0.05$), no significant variation occurs thereafter.

This implies that the incidence of glucose loss reached its maximum between the 21st. and 30th week of pregnancy, and this was maintained until delivery. It is interesting to note, however, that 10 out of 34 cases remained negative throughout and 3 remained consistently positive. Ten of the remaining 21 cases who had previously registered positive reductions for urinary glucose, became negative from 36 weeks until delivery.

In the urine specimens examined, reductions were found to be caused by the presence either of an individual sugar or by groups of 2 or 3 sugars acting concurrently. Chromatography demonstrated 5 varieties, viz., glucose, lactose, galactose, pentose and fructose.

Fructose contributed to relatively few reductions so that valid inference is limited. It is probably safe to assume that the 6 fructose reductions, 3 of which were obtained from urine

specimens of the same individual, resulted from a dietary course of that sugar. Of these there are many, since fructose occurs as free hexose chiefly in sweet fruit and honey. Apples are relatively rich in laevulose and as a component with glucose of the disaccharide saccharose, it is found in relatively large amounts in sugar beets and in sugar cane.

Pentose was found on 4 occasions only (3 cases). One of the women gave a history of vomiting. Soskin and Levine (1955) claim that the 5 carbon atom sugars are much more important as part of the machinery of the body in contrast to the important energy materials, the hexoses. While admitting that they may possibly contribute to the pentose content of the tissues when ingested in combined form (e.g. riboflavin and nucleotides), these authors claim that uncombined pentoses are not utilized when ingested, but are eliminated in the urine and faeces.

Lactose excretion increased in frequency as pregnancy advanced. From the 20th week, this

was the reducing sugar most frequently present, although the concentration never exceeded 50 mg. per 100 ml. urine in the present series.

Galactose like glucose and lactose was detected before the 20th week, and although not nearly so high, its incidence would appear to run parallel to that of lactose.

Glucose. Urinary glucose was detected in over 75 per cent of the women in this series. In the majority there was a significant increase in the incidence of excretion of this sugar from the 21st. to the 30th week of gestation. In general, this maximal value was maintained during the last four weeks of pregnancy.

During this investigation the opportunity was taken to examine the influence of both age and parity in the incidence of sugar excretion in normal pregnancy urine. It has been suggested that both of these factors may influence carbohydrate tolerance. In the human subject in general, tolerance is believed to decrease with age, while for some time it has been suggested

that increasing parity and tolerance may also bear an inverse relationship to one another (Rosenberg 1924 ; Lambie 1926).

In this series of cases, the ages ranged from 17 to 40 years. It was not, however, possible to establish any significant variation in the incidence of urinary sugar attributable to this factor, owing to the generally high frequency of its excretion.

The same was true regarding the influence of parity. In this connection, it might be pertinent to mention that this finding is consistent with experience gained during the later stages of these studies. In the course of these, it has been possible to observe a few of the patients in a subsequent pregnancy. As a result, it has been noted that although glycosuria of a degree sufficient to merit full investigation by standard oral tolerance tests may be detected in one gestation, this need not necessarily be a recurrent feature in a further pregnancy.

SUMMARY OF RESULTS.

1. The urinary excretion of sugar was studied in 18 primigravid and 16 parous women from before the 21st. week of pregnancy.

Only one subject completed gestation without having sugar detected in her urine.

2. In descending order of frequency, the sugars identified by paper chromatography in the urine specimens of the other subjects were lactose, glucose, galactose, fructose and pentose.

These occurred either individually or in groups of two or three.

3. There was a progressive increase in the incidence of lactose excretion from the 20th week until delivery.

There was a parallel increase in the excretion of galactose, although this sugar was detected much less frequently than lactose.

4. Glucose was detected in the urine of over 75 per cent of the cases.

The maximal number of cases excreting glucose was reached in the period from 21 to 30 weeks.

The majority of these continued to excrete glucose until delivery.

5. Neither age or parity appeared to have any influence on the excretion of sugar.

COMMENTARY.

The results of this preliminary investigation suggest that the incidence of glycosuria is high in normal pregnancy.

Its occurrence seems to be independent of age or parity.

Furthermore, since no specific diet was prescribed, the glycosuria would appear to be independent of any particular type of diet.

These patients were healthy in all repsects.

It is thus reasonable to suggest that some factor or combination of factors, influences carbohydrate metabolism in the gravid state.

Lactose. The pattern of lactose excretion is almost certainly related to breast development and function.

Galactose and Glucose. With regard to the excretion of these sugars, there are the following possibilities:-

(a) Placental function. It is possible that part of the sugar output may be derived as breakdown products of such substances as gonadotrophins. Morris (1955) in discussing the chemical and physico-chemical properties of chorionic gonadotrophin, describes it as a glyco-protein of high carbohydrate content. This includes a hexose identified by Gurin et al. (1940) as galactose. The kidney during excretion may liberate the carbohydrate as the hormone is broken down. Alternatively, urinary galactose may represent an uncombined precursor, or a breakdown product, of the lactose molecule.

The significant increase in the incidence of glucose excretion which takes place after the 20th week of pregnancy may be related to the fact that the placenta becomes fully functional at that time. This increase appears to be maintained throughout the period of placental maturity, which persists in the majority of women until just before delivery. However, of the 34 cases, 10 who had previously passed

glucose in their urine ceased to do so after the pregnancy had reached the 36th week. Although their records afford no evidence to support such a hypothesis, it might well be that placental senescence had begun unusually early in this minority. It must be admitted, however, that the possibility of there being such an index of placental sufficiency has never, to this writer's knowledge, been investigated.

(b) Blood sugar levels may be abnormal.

(c) Renal handling of the sugars may be altered in pregnancy.

Both (b) and (c) have been investigated later in these studies.

It is well known that sugar is commonly found in the urine of pregnant women. The high incidence of its occurrence, as shown in this preliminary investigation, is perhaps less generally appreciated. In view of the successful out-come of the pregnancies just studied, it is obviously unnecessary (if not, indeed, harmful) to restrict carbohydrate in

cases of simple glycosuria. This view is in agreement with that expressed by Donald (1959) who states that such cases require no treatment.

The present findings indicate that there is a change in the metabolism of glucose from the 21st. to 30th weeks which is maintained in late pregnancy. This may explain the experience of those clinicians such as Wilkerson & Remein (1957) who have found that those cases pronounced simple glycosurias in the early months of gestation must be retested in later pregnancy in order to be certain of excluding diabetes. Attention must be paid to this factor in treating the established diabetic. Furthermore, these results would suggest that stabilisation cannot be achieved on the basis of urinalysis.

In view of the obscure nature of the mechanism of glycosuria in normal pregnancy, a more detailed investigation seemed to be necessary. Because of this and its obvious importance to the practice of obstetrics, it was decided to investigate

carbohydrate tolerance in normal gravid women by means of a series of "loading" tests.

SECTION II.

THE ASSESSMENT OF CARBOHYDRATE TOLERANCE BY MEANS OF INTRAVENOUS "LOADS".

The object of the present series of investigations was to study the utilization and excretion of carbohydrate in normal pregnancy by means of administering test loads of sugar. Further, to study carbohydrate metabolism under the same experimental conditions in cases of (a) twin pregnancy, and (b) in cases of pregnant women who had shown glycosuria prior to the experimental loading. Finally, to compare and contrast the results obtained in the normal group with those in the other groups.

Twin pregnancy was examined because the preliminary investigation had indicated a connection between placental development and the impairment of carbohydrate tolerance. It was considered possible that the larger placental area of multiple pregnancy might cause greater impairment.

The cases of glycosuria had been detected on routine urinalysis at the ante-natal clinic when

their urinary sugar concentrations had ranged from 75 mg. to 200 mg. per 100 ml. urine. The glycosuria had been diagnosed as of renal origin following standard oral glucose tolerance tests. These cases were studied with a view to determining whether their carbohydrate metabolism differed substantially from that of normal pregnant women.

To achieve these aims it was necessary, therefore, to perform a standardised test on four groups of subjects:-

(1) Normal non-gravid women of child-bearing age.

(2) Normal gravid women.

(3) Cases of twin pregnancy.

(4) Cases of "renal glycosuria".

The load effects obtained in groups 1, 3 and 4 were then compared in turn with those obtained in group 2.

It will be appreciated that considerable importance attaches to the method of loading. In the present instance it was decided to load by means of a single intravenous injection of

dextrose.

The intravenous route of administration has been employed by numerous workers, and Tunbridge and Allibone (1949) describe the individual methods which have been adopted by some 37 teams between 1913 and 1938. Since then, intravenous glucose tolerance tests have been described by several authors including Johnson and Bosnes (1948), Duncan (1956a) and Marazzini (1957) and the advantages over the oral route are now generally agreed:-

1. The oral tests depend upon the degree of absorption of the glucose by the alimentary canal. This introduces definite and uncontrollable factors, since the two processes of absorption and disposal tend to be coincident over an indefinite period.

2. Alterations in the gastro-intestinal tract peculiar to pregnancy may complicate absorption.

This was of particular importance in the present study where it was necessary to compare

a gravid with a non-gravid series of patients.

3. Following intravenous administration, the maximal hyperglycaemia occurs rapidly and the subsequent fall in blood sugar is uninfluenced by simultaneous absorption.

For these reasons it has been suggested that narrower limits of normality can be imposed when the sugar is injected intravenously (Stowers, 1961).

Although there has been general agreement on principle, there has been a wide diversity of opinion with regard to procedure. Duncan (1956a) comments in particular on the lack of uniformity in the loading dose of sugar and in the evaluation of results. With regard to the latter, he himself prefers to express tolerance as an "increment index", while Tunbridge and Allibone (1940) made their criterion the time taken for the blood sugar to regain fasting values. Crawford (1938) used the time taken to reach the value of 100 mg. per 100 ml. blood, and Lozner et al. (1941) took the 2-hour value as their index. Quite different standards were those adopted by

Jorgenson (1927) and Ross (1938) who measured the area under the observed blood sugar curve.

Similarly, there has been individual variation in the selection of times for the withdrawal of blood. Duncan (1956a) took his first sample four minutes after completion of the injection, since he estimated that complete mixing of sugar with the blood would have occurred by then. This is not in agreement with the views of Turner and Allibone (1940) who found that blood sugar levels were maximal one minute after the end of the injection. (These authors, however, reported great differences in this level between individual subjects). Johnson and Bosnes (1948) appear to have overcome this difficulty by waiting 15 minutes after the end of the injection before taking their first blood sample.

In the present study, a load of 20 g. of 50 per cent dextrose was employed. This dose is smaller than that used by many workers, but it was felt that nothing was to be gained by the injection of larger amounts of sugar. On the contrary, the lower dosage had the advantage of producing

appreciable changes in blood sugar levels which were, at the same time, closer to the normal physiological variations than those which would have resulted from higher loads. It was hoped that this would add to the practical value of the subsequent observations.

The first blood sample, following the fasting specimen, was taken 30 minutes after the end of the injection. Further specimens were obtained at intervals of 30 minutes to give a coverage of 2½ hours. These times were chosen so that the intermediate and more remote post-injection blood sugar levels and their effects on urinary sugar output could be observed.

In addition, the technique which is later described in detail (page 41) differed from those already mentioned, in that all the specimens of urine were analysed qualitatively by means of paper chromatography.

Finally, this study was essentially a comparison of the effects of carbohydrate administration on four groups of patients who were subjected to the same experimental conditions.

Because of this, it was possible to evaluate the results statistically without adopting any of the individual criteria advocated by the authors mentioned previously.

Although from clinical considerations the investigation was concerned with the influence of pregnancy on the metabolism of glucose particularly, it was felt important to decide whether any possible changes were specific for that hexose alone. To do this, it was necessary to obtain supplies of another sugar which were suitable for intravenous administration. Furthermore, it was a pre-requisite that the hypertonic solution of carbohydrate should have no harmful effect upon the renal tubules. It is now generally accepted that such effects are liable to occur when, for example, the disaccharide sucrose is injected in this manner (Anderson 1948 ; Morard et al., 1956). While Cappel (1958) considers that the intense hydropic degeneration so produced in the renal tubule cells is temporary and unlikely to result in serious harm, it will be appreciated, that any induced changes, even of a

temporary nature, are likely to invalidate load tests.

In the end it was decided to use fructose (d-laevulose) which not only satisfied the requirements mentioned above, but offered certain additional advantages:-

In 1920 Maclean and de Wesselow found that the ingestion of fructose, in contrast to other sugars such as glucose, produced little if any increase in total blood sugar. This is now understood to be caused by the rapid conversion of laevulose to glycogen by the liver. (The works permitting such a conclusion, including those of Mann and Magath (1922) ; Cori (1925) and Kimball (1932), have been reviewed and commented upon by Stewart et al. (1938). These authors, while conceding the possible importance of direct utilization by the tissues, concluded that laevulose metabolism takes place to a very large extent in the liver).

When the fructose is administered intravenously,

in contrast to the oral route, there occurs a significant rise in total blood sugar, although it is still considerably less than that effected by intravenous glucose. For practical purposes this may be expressed simply:-

Total Blood Sugar = Fasting Blood Sugar + Glucose
(Largely glucose) (Converted from fructose)
+ Fructose.

The work of Gammeltoft and Kjerulf-Jensen (1943) suggests that raising the total blood sugar mass by the introduction of fructose would provide competition to the glomerular filtration and re-absorption of glucose. These authors concluded from animal experiments, as well as from observation on the human subject, that fructose and glucose are actively re-absorbed from the proximal renal tubules. While tentatively suggesting that there are phosphorylating and dephosphorylating enzyme systems specific for each hexose, they advanced the view that glucose and fructose compete with each other as phosphate acceptors from the common phosphate donor adenosine triphosphate.

In view of these considerations, it was decided to perform a fructose load test, in addition to the glucose test, in each of the subjects of the present investigation. It was hoped that the introduction of a competitor to the renal excretion and re-absorption of glucose might help to accentuate any pre-existing impairment which pregnancy had occasioned in that mechanism.

METHOD AND MATERIALS.

A series of 10 non-gravid women whose ages ranged from 18 years to 38 years was obtained in the Royal Samaritan Hospital for Women. The cases were selected to exclude endocrinological abnormality and only those of good general health and nutrition were chosen.

On admission they were given the ordinary hospital diet which included approximately 350 g. carbohydrate daily.

The patients were fasted from 10 p.m. on the evening preceding the test. The following morning, a specimen of the "fasting" urine was obtained and immediately prior to the commencement of the test

at 9 a.m., a self-retaining catheter was introduced to the bladder which was then emptied. The entire volume of urine was discarded and the catheter was secured by means of a spigot.

The "fasting" blood sample was then obtained and without withdrawing the No. 2 serum needle from the vein in the antecubital fossa, 40 ml. of 50% (i.e. 20g.) dextrose solution were introduced in 1½ - 2 minutes, by means of a 50 ml. all-glass syringe. Further blood specimens were obtained from the opposite median basilic vein at 30-minute intervals until conclusion of the test 150 minutes later. The blood specimens were retained in fluoride bottles. At 75 and 135 minutes following the injection, the bladder was drained but not washed out. This gave the first and second urine specimens.

Thus from each test there were six blood specimens including the "fasting" blood, and three urine specimens including the "fasting" urine.

On each blood specimen total blood sugar was estimated by the Nelson (1944) adaptation of the Somogyi method. Each urine specimen was tested

for sugar by "Clinitest" tablets and, where a reduction was registered, by the method of paper chromatography. (Appendix A).

After an interval of two or three days the test was repeated using 40 ml. 50% (approx.) fructose solution. This was prepared by the addition of 8 ml. sterile water to 32 ml. of 62.5% "Laevosan" concentrate (Calmic Ltd.), thus giving 20 g. carbohydrate in 40 ml. solution as for the dextrose tolerance test.

Parallel tests were performed on 10 cases of normal pregnancy, 10 cases of twins, and 10 cases of renal glycosuria.

COMPLICATIONS AND SIDE EFFECTS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF SUGARS.

The advantages of assessing carbohydrate tolerance by the intravenous administration of sugars have been discussed earlier. It must be appreciated, however, that previous workers have reported complications following the intravenous injection of dextrose.

Most frequent among these have been pyrexia, rigors, general malaise and headaches.

Jorgensen (1927) noted pyrexia in four of his first 75 cases. Thereafter, he employed freshly distilled water for the dextrose solutions and no further complications occurred. From this one might be tempted to assume that the modern techniques employed in the preparation of sterile solutions of sugar, might provide immunity from such complications. This, however, is not entirely true.

Duncan (1956a) reported "slight ill effects or complications" following the intravenous administration of 25 g. 50% dextrose; but when the dose was increased to 50 g. undesirable side effects were encountered. These included considerable pyrexia with malaise, headache and generalised body and joint pains which lasted for about 12 hours on the day following the test. In addition, three of his initial five cases developed venous thrombosis. As a result of this complication, Duncan decided not only that a dose of 50 g. dextrose was contra-indicated, but also that the simultaneous injection of heparin was desirable as a prophylactic measure.

Symptoms similar to those already described have been reported by Turner and Allibone (1949), who stressed the outstanding feature of their having no complications in those of their patients who were confined to bed. This finding was rendered more significant by the fact that 15 of their out-patient subjects had complications including pain in the arm, phlebitis, malaise, headache, nausea and pyrexia. Some of these did not occur until several days had elapsed.

All the subjects of the present investigations were admitted to hospital because previous experience had indicated that this ensured much more satisfactory control. They were permitted to be ambulant in the periods immediately preceding and after the tests. Prophylactic heparin was not employed, and indeed proved to be quite unnecessary. There were no complications in the gravid or non-gravid patients from the 40 tests employing the relatively small dose of 20 g. 50% dextrose.

In contrast, when the corresponding 40 fructose load tests were performed on the same

patients, a significant number experienced side effects. While it is true that these were minimal in the pregnancy group (of whom only nine were affected), they were well marked in all of the non-gravid subjects. Indeed the striking difference in the degree of response shown by the gravid on the one hand, and the non-gravid cases on the other, merits a description of the behaviour of both groups.

It will be recalled that the fructose load of 20 g. (40 ml.) 50% laevulose was injected intravenously during a period of 1½ - 2 minutes.

Those gravid cases who experienced side effects, complained of "a feeling of warmth from head to toes" after an interval of 2 - 3 minutes. There was an associated, very transient, feeling of faintness, but there were no other symptoms. Meanwhile the observer could readily discern a suffusion of the face suggestive of a peripheral vasodilatation producing a curiously mottled appearance. This sign, which persisted for some 7 - 10 minutes,

was observed in none of the non-gravid subjects. In these, the following symptoms occurred in varying degree:

After an interval of 2 - 5 minutes following the injection of fructose, the non-gravid women complained of a feeling of warmth just as the gravid patients had done. In some there was a feeling of lassitude and faintness, described by one subject as being "like getting gas at the dentist's". The most dramatic and constant side effect was a sensation experienced in the epigastrium. Each patient indicated with one finger a point situated in the midline just below the xiphisternum. This was the seat of a "peculiar sensation", in some remaining like a sense of pressure, in others developing into quite severe pain within five minutes of its onset. None of these symptoms persisted for longer than 12 minutes, and apart from a transient rise of some 20 beats per minute in pulse rate with an associated moderate tachypnoea, no objective signs were elicited in these cases.

It has been suggested that these side effects, which did not occur in any of the subjects following the administration of dextrose, may be due to modifications in the intermediary metabolism of fructose, particularly in the liver (Williams, 1962). While the relative immunity of the gravid subjects is of particular interest, it must be emphasized that the experience gained suggests that these side effects are of no serious consequence even in the non-gravid. Certainly they do not contra-indicate the intravenous administration of laevulose. Indeed, although this was not possible in the course of the investigations because of the necessity of the tests being standardised, subsequent experience has shown that these side effects may be eliminated or rendered minimal by reducing the speed of fructose administration.

RESULTS.

The results of the 80 tests are presented in Tables 6 - 9 and the corresponding mean

TABLE 6.

NON-GRAVID DEXTROSE/FRUCTOSE LOADS.

Total Blood Sugar mg. per 100 ml.

Case No.	Age	Parity	I.V. Sugar.	Time (mins). 0	30	60	90	120	150
1.	18.	0.	D	77	130	93	72	68	69
			F	77	104	71	70	70	72
2.	22.	0.	D	70	145	83	68	78	78
			F	76	115	81	81	83	80
3.	23.	1.	D	83	140	81	68	60	70
			F	86	116	97	76	70	72
4.	25.	1.	D	85	192	154	104	81	60
			F	85	104	93	76	74	78
5.	27.	1.	D	88	152	107	81	59	59
			F	82	126	89	81	79	83
6.	32.	2.	D	87	147	107	85	77	79
			F	92	116	92	88	83	81
7.	33.	2.	D	85	171	127	93	74	64
			F	82	91	83	82	81	84
8.	35.	0.	D	80	137	90	67	60	66
			F	83	128	89	80	76	85
9.	22.	0.	D	76	140	77	70	65	72
			F	78	118	83	84	84	83
10.	33.	1.	D	88	166	127	92	72	68
			F	90	126	103	95	91	91

TABLE 7.

NORMAL PREGNANCY - DEXTROSE/FRUCTOSE LOADS.

Total Blood Sugar mg. per 100 ml.

Case No.	Age	Gravida	Weeks Pregnant		Time (mins.) 0	30	60	90	120	150
11	39	4	40	D	79	124	95	78	77	76
					77	108	96	87	78	80
12	31	2	34	D	80	133	92	83	72	75
				F	80	94	87	83	77	77
13	30	3	33	D	70	120	81	70	66	70
				F	80	97	80	80	72	70
14	27	7	34	D	82	104	73	62	68	68
				F	70	77	74	73	69	65
15	27	3	40	D	72	137	102	83	73	70
				F	68	99	84	77	70	70
16	24	2	33	D	86	126	99	79	72	75
				F	90	114	84	80	78	83
17	24	3	39	D	65	114	86	68	56	60
				F	67	91	78	71	68	65
18	23	1	34	D	80	157	115	87	82	68
				F	77	95	87	82	73	77
19	22	1	34	D	80	145	99	74	73	73
				F	79	103	82	75	78	80
20	21	3	40	D	77	120	85	68	64	67
				F	76	98	77	75	67	72

TABLE 8.

TWIN PREGNANCY - Dextrose/Fructose Loads.

Total Blood Sugar mg. per 100 ml.

Case No.	Age.	Gravida.	Weeks Pregnant	I.V. Sugar.	(Time mins.) 0	30	60	90	120	150
21.	23.	1.	38.	D	77	112	83	72	70	74
				F	80	93	80	81	77	79
22.	30.	5.	30.	D	83	110	83	73	75	75
				F	81	98	84	80	81	79
23.	23.	2.	23.	D	70	103	80	70	60	66
				F	71	90	80	76	70	71
24.	31.	3.	31.	D	77	114	83	73	65	63
				F	70	86	74	72	72	70
25.	22.	3.	34.	D	75	113	93	72	66	68
				F	68	91	72	65	60	59
26.	25.	1.	38.	D	70	108	90	69	64	58
				F	73	89	71	67	64	64
27.	25.	1.	36.	D	67	111	78	63	63	64
				F	60	78	70	60	66	63
28.	30.	3.	37.	D	62	129	101	72	55	57
				F	71	92	71	66	66	62
29.	26.	1.	26.	D	77	126	98	74	70	74
				F	77	97	83	78	74	69
30.	34.	1.	35	D	66	98	68	54	55	54
				F	62	84	62	56	54	56

TABLE 9.

GLYCOSURIA - Dextrose/Fructose Loads

Total Blood Sugar mg. per 100 ml.

Case No.	Age	Gravida	Weeks Pregnant	I.V. Sugar	Time (mins.) 0	30	60	90	120	150
31	28	1	36	D	82	152	108	79	70	72
				F	79	111	105	87	81	79
32	36	4	17	D	76	149	81	68	65	62
				F	85	96	84	76	74	74
33	24	1	32	D	87	120	90	77	80	80
				F	81	109	87	84	79	79
34	29	4	34	D	80	135	100	77	74	75
				F	80	98	78	72	77	77
35	21	1	29	D	81	110	85	77	72	77
				F	81	97	82	76	78	78
36	22	1	34	D	70	116	97	75	66	61
				F	80	100	85	80	78	80
37	28	4	28	D	92	136	126	92	87	85
				F	87	104	90	88	85	80
38	42	10	39	D	73	110	83	71	62	60
				F	66	85	71	70	62	65
39	27	1	33	D	85	131	97	80	70	68
				F	78	99	78	76	75	69
40	40	3	32	D	85	135	115	96	83	80
				F	81	93	87	83	72	76

FIG. 2 I.V. DEXTROSE LOADS — MEAN VALUES OF TOTAL BLOOD SUGAR.

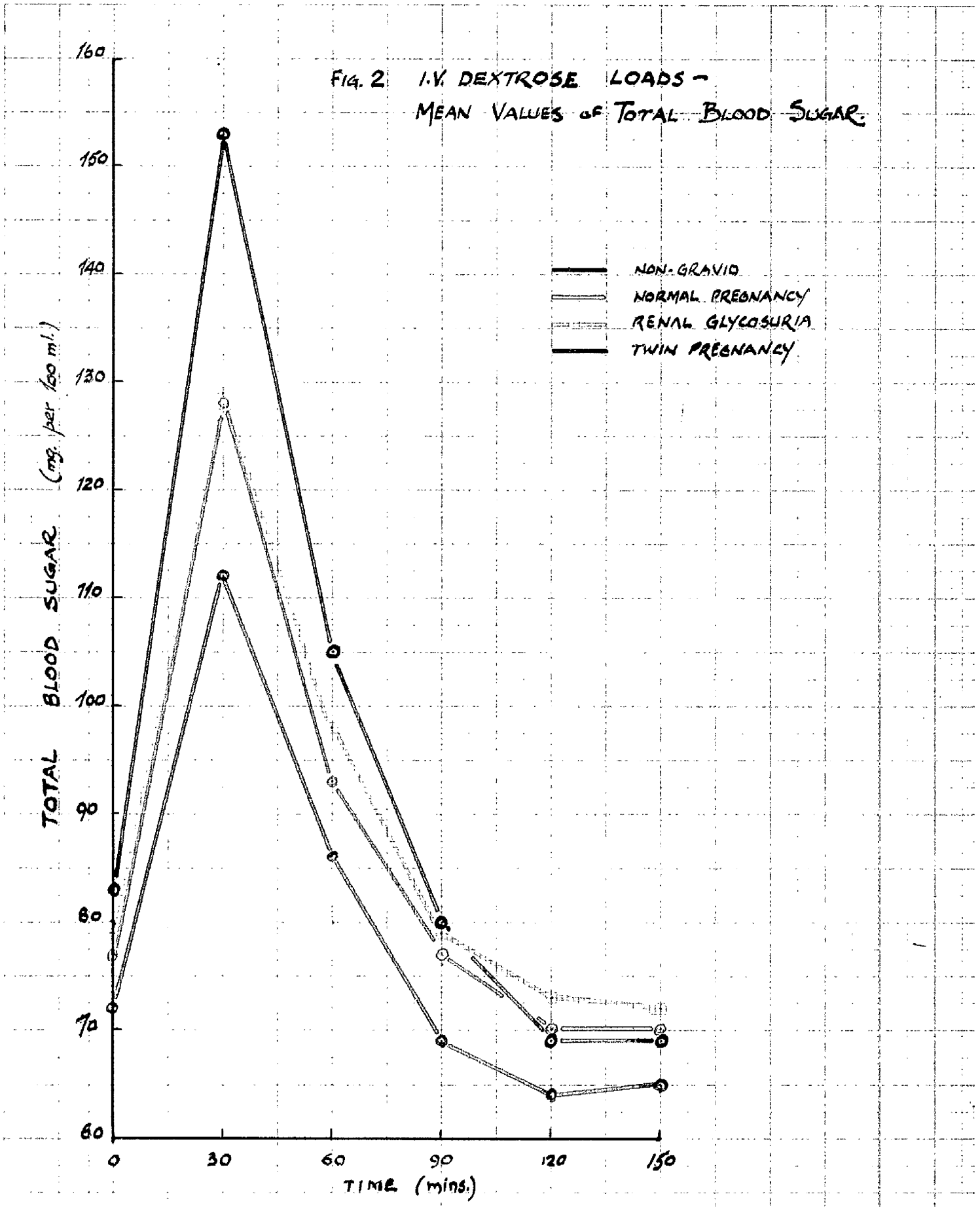
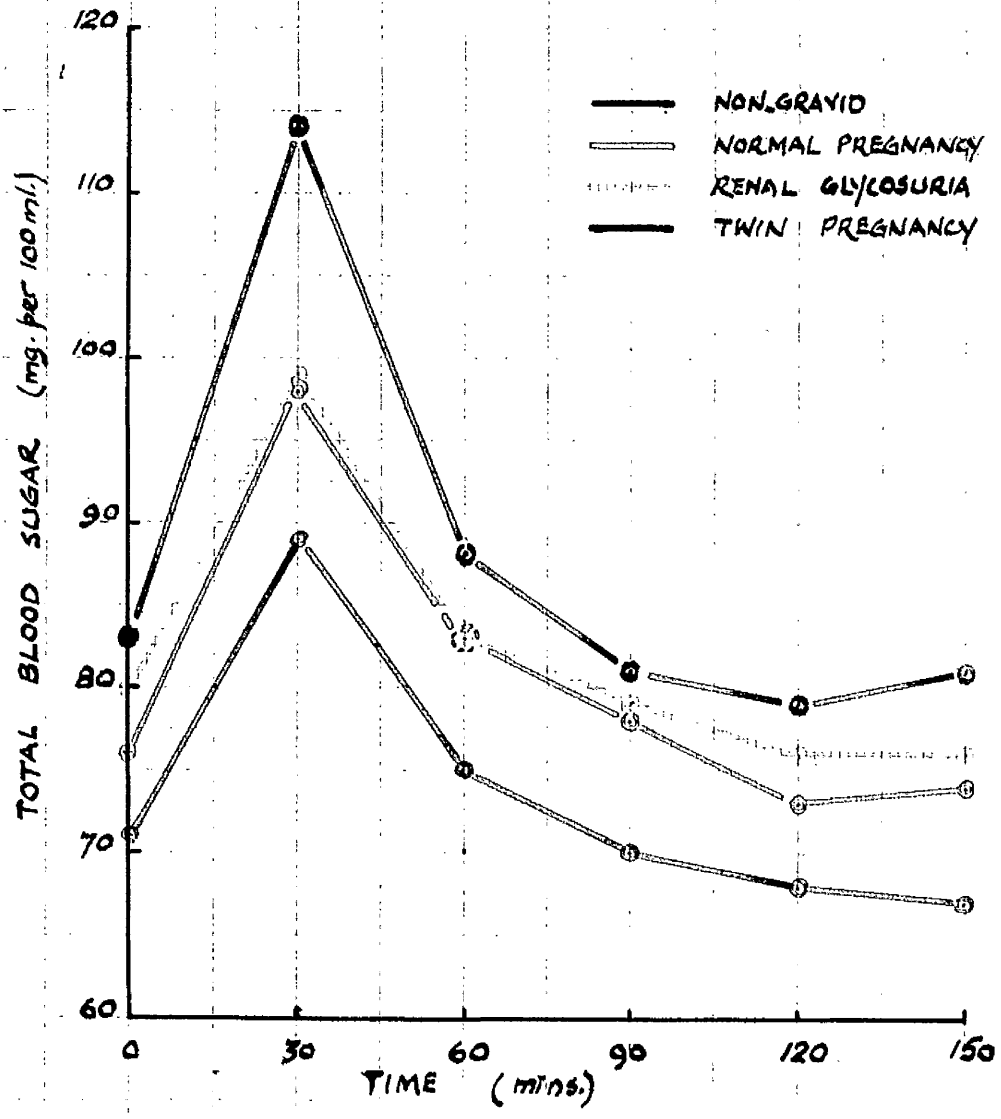


FIG 3. I.V. FRUCTOSE LOADS —

MEAN VALUES OF TOTAL BLOOD SUGAR.



values for each series of glucose and fructose tests are illustrated graphically in figs. 2 and 3.

STATISTICAL ANALYSIS.

(a) GLUCOSE

Table 10.

GLUCOSE : Total Blood Sugar mg. per 100 ml.

Time	Non-gravid	Normal Pregnancy	Twins	Glycosuria
0	82.7	77.1	72.4	81.1
30	152.8	128.0	112.4	129.4
60	104.6	92.7	85.7	98.2
90	80.0	75.2	69.2	79.2
120	69.4	70.3	64.3	72.9
150	68.5	70.2	65.3	72.0

Table 11.

Combined Fasting Blood Sugar Mean Values.

Group	Mean	Standard Deviation	Number of Observations
Non-gravid	82.90	4.85	20
Normal Pregnancies	76.75	6.39	20
Twins	71.85	6.53	20
Glycosuria	80.45	6.01	20

An analysis of variance leads to the conclusion that for the combined set of observations, the

Non-gravid is highly significantly different from Normal Pregnancy; Twins yield a significant difference from Normal Pregnancy; Glycosuria is bordering on being significantly different from Normal Pregnancy.

For times 30, 60, 120 minutes it is necessary to consider each time separately for glucose and fructose. This gives the following results:-

(1) Glucose.

Time 30:- The Normal Pregnancy is 128.0. A difference of 7.2 is required for significance. Thus the Non-gravid is significantly higher, the Twins are significantly lower.

Time 60:- The Normal Pregnancy is 92.7. Again, a difference of 7.2 is required for significance. Thus the Non-gravid is significantly higher. The Twins are bordering on being significantly lower.

Time 90:- The Normal Pregnancy is 75.2. No significant differences from this value.

Time 120:- Again no significant differences from the Normal Pregnancy.

Time 150:- As for time 120. No significant differences.

11) Fructose.

Table 12.

FRUCTOSE : Total Blood Sugar (mg. per 100 ml).

MEAN VALUES.

Time	Non-gravid	Normal Pregnancy	Twins	Glycosuria
0	83.1	76.4	71.3	79.8
30	114.4	97.6	89.8	99.2
60	88.1	82.9	74.7	84.7
90	81.3	78.3	70.1	79.2
120	79.1	73.0	68.4	76.1
150	80.8	73.9	67.2	75.9

Time 30:- The Normal Pregnancy is 97.6. A difference of 3.9 is required to establish significance. Thus the Non-gravid is significantly higher, the Twins are significantly lower.

Time 60:- The Normal Pregnancy is 82.9. Any mean outside the interval 82.9± 3.0, i.e. 79.0 to 86.8 is significantly different. Thus the Non-gravid is significantly higher, the Twins significantly lower.

Time 90:- The Normal Pregnancy is 78.3. Any mean outside the interval 78.3± 3.9 i.e. from 74.4 to 82.2 is significant. Thus the Twins are significantly lower.

Time 120:- The interval is 73.0± 3.9 i.e. from 69.1 to 76.9. Thus Non-gravid is significantly higher and Twins significantly lower.

Time 150:- The interval is 73.9± 3.9 i.e.from 70.0 to 77.8. Thus Non-gravid is significantly higher and Twins significantly lower.

b) Urinary Sugar.

1) GLUCOSE

Table 13.

Urinary Sugar (mg. per 100 ml.) Mean Values.

Specimen.	Non-gravid.	Normal Pregnancy	Twins	Glycosuria.
Fasting.	0.0	15.0	15.0	27.5
1st.	80.0	60.0	47.5	112.5
2nd.	35.0	27.5	40.0	65.0

(Since the Non-gravid passed no sugar in the Fasting, only the 1st. and 2nd. specimens are analysed).

First Urine. The Normal Pregnancy is 60.0. Any mean which deviates at least as much as 25.2 from 60.0 is significantly different. Thus Glycosuria is significantly higher.

Second Urine. The Normal Pregnancy is 27.5. Any mean outside the interval 27.5± 25.2 i.e. from 2.3 to 52.7 is significantly different. Thus Glycosuria is significantly higher.

11) FRUCTOSE.

Table 14.

Urinary Sugar (mg. per 100 ml.) Mean Values.

Specimen.	Non-gravid.	Normal Pregnancy	Twins	Glycosuria.
Fasting.	0.0	15.0	15.0	22.5
1st.	80.0	77.5	70.0	107.5
2nd.	50.0	42.5	47.5	45.0

First Urine. The Normal Pregnancy is 77.5. Any mean outside the interval 77.5± 13.8, i.e. from 63.7 to 91.3 is significantly different. Thus Glycosuria is significantly higher.

Second Urine. The interval is 42.5± 13.8, i.e. from 28.7 to 56.3. No significant differences from Normal Pregnancy.

To consider these results further:-

1. Glucose.

a) Non-gravid compared with Normal Pregnancy.

Sugar was absent from all the fasting urine specimens obtained from the non-gravid subjects, although the fasting blood sugar levels were significantly higher than those of the normal pregnancy group. The majority of the fasting urine specimens obtained from the latter yielded

a positive reduction. (This was also true of the cases of twins and glycosuria).

At 30 and 60 minutes after the intravenous injection of glucose, total blood sugar levels were significantly higher in the non-gravid. Nevertheless, the first urine specimens, which were collected at 75 minutes, showed no difference in sugar concentrations from those of normal pregnancy.

Although the blood sugar in both groups approximated to fasting levels throughout that period of the tests which followed the first urine collections, it was observed that each continued to excrete sugar.

Thus, the final urine specimens, collected at 135 minutes, showed no significant difference in the concentrations of sugar excreted during this period of relatively low blood sugar levels.

b) Twin Pregnancy compared with Normal Pregnancy.

There was no difference from the normal pregnancy cases with regard to urinary sugar concentrations despite the fact that the fasting, 30 minute and 60 minute blood sugar levels were

lower in the cases of twin pregnancy.

c) Glycosuria compared with Normal Pregnancy.

The concentrations of urinary sugar were significantly higher in the cases of glycosuria, although the blood sugar levels showed no appreciable difference from those of normal pregnancy.

11) Fructose.

Following the administration of fructose, the total blood sugar levels for each of the four groups were inferior to those which had been produced by glucose in the corresponding group. This result was to be expected from theoretical considerations (page 39).

More particularly, the total blood sugar levels of the fructose tests closely paralleled those of glucose for each type of subject (Figs. 2,3).

Indeed, it was possible to determine statistically significant differences in blood sugar levels between normal pregnancy on the one hand, and non-gravid cases and twins on the other, at every interval of the fructose tests. That is

to say, those differences which had been established in the more immediate and intermediate intervals of the glucose load tests were found, in addition, to be maintained throughout the more remote post-infusion periods of the fructose load tests.

The cases of glycosuria, as in the glucose tests, passed mugh higher concentrations of urinary sugar although the corresponding blood sugar values did not differ from those of the normal pregnancy group.

III. Chromatography.

The results of the sugar chromatograms are incorporated in Tables 15,16,17,18, in which, for conciseness, the names of the individual sugars are abbreviated-Glucose (G), Lactose (L), Galactose (Gal.), Fructose (F).

The notations Tr., +, ++, +++, ++++, are intended to signify the intensity of the staining reaction of any given sugar on the chromatogram and, in particular, to show the relative amounts of the individual sugars when two or more are

TABLE 15.

NON-GRAVID - Urinary Sugar by "Clinitest" (mg. per 100 ml.) and Chromatography.

Case No.	DEXTROSE. Fasting Clin.	Fasting Chromy.	1st. Specimen Clin.	1st. Specimen Chromy.	2nd. Specimen. Clin.	2nd. Specimen. Chromy.	FRUCTOSE. Fasting. Clin.	Fasting. Chromy.	1st. Specimen Clin.	1st. Specimen Chromy.	2nd. Specimen. Clin.	2nd. Specimen. Chromy.
1.	-	-	50	G	25	G	-	-	100	F	25	F
2.	-	-	75	G	25	G	-	-	50	F	50	F
3.	-	-	50	G	50	G	-	-	75	F	50	F
4.	-	-	75	G	50	G	-	-	75	F	50	F
5.	-	-	200	G	-	-	-	-	100	F	25	F
6.	-	-	50	G	50	G	-	-	50	F	-	-
7.	-	-	50	G	50	G	-	-	100	F	75	F
8.	-	-	-	-	-	-	-	-	75	F	50	F
9.	-	-	50	G	25	G	-	-	75	F	75	F
10.	-	-	200	G	75	G	-	-	100	F	100	F

TABLE 16.

NORMAL PREGNANCY - URINARY SUGAR BY "CLINITEST" (mg. por 100 ml.) AND CHROMATOGRAPHY.

Case No.	DEXTROSE. Fasting. Clin.	Chromy.	1st. Specimen Clin.	Chromy.	2nd. Specimen Clin.	Chromy.	FRUCTOSE. Fasting. Clin.	Chromy.	1st. Specimen Clin.	Chromy.	2nd. Specimen Clin.	Chromy.
11.	25	G L	50	G++ L	25	G L+	50	L	75	L	50	L
12.	25	G	75	G	75	G++ L	50	G+ L	100	G L F++	75	G L F+
13.	25	G	50	G++ L	75	G+++ L	25	G+ L	25	L+ F	25	G L
14.	-	-	50	G+	-	-	25	G	50	F	25	G
15.	25	G L	100	G+++ L	25	G L	-	-	75	F	25	G L
16.	-	-	50	G	-	-	-	-	75	F	25	L
17.	25	L	75	G+ L	25	L	-	-	100	F	50	F
18.	25	G	50	G	25	G	-	-	100	F	50	F
19.	-	-	50	G++ L	-	-	-	-	75	L F++	50	F
20.	-	-	50	G+ L	25	G+ L	-	-	100	L F++	50	L

TABLE 17.

TWIN PREGNANCY - Urinary Sugar by "Clinitest" (mg. per 100 ml.) and Chromatography.

Case No.	DEXTROSE. Fasting. Clin.	Fasting. Chromy.	1st.Specimen Clin.	1st.Specimen Chromy.	2nd.Specimen. Clin.	2nd.Specimen. Chromy.	FRUCTOSE. Fasting. Clin.	Fasting. Chromy.	1st.Specimen. Clin.	1st.Specimen. Chromy.	2nd.Specimen Clin.	2nd.Specimen. Chromy.
21.	25	L	50	G++ L	25	G+ L	25	L	50	F	50	F
22.	25	L	50	G L	50	G+ L	25	L	100	F	50	F
23.	25	G L	75	G++ L	50	G+ L	25	L	75	G+ L F	50	G+ L F+
24.	25	G	50	G+ L	75	G++ L+	-	-	25	F+	25	F+
25.	25	L	-	-	50	G	25	G L	100	G+ L F++	50	L F+
26.	-	-	50	G	25	G L	25	L	75	L F++	75	L. F++
27.	25	G L	100	G++ L	75	G+ L	25	G L	75	F	50	F
28.	-	-	50	G+ L	25	G L	-	-	75	F	50	L F+
29.	-	-	50	G+ L	25	G L	-	-	75	F	50	L F+
30.	-	-	-	-	-	-	-	-	50	F	25	F

TABLE 18.

GLYCOSURIA - Urinary Sugar by "Clinitest" (mg. per 100 ml.) and Chromatography.

Case No.	DEXTROSE. Fasting. Clin.	Fasting. Chromy.	1st. Specimen. Clin.	1st. Specimen. Chromy.	2nd. Specimen Clin.	2nd. Specimen Chromy.	FRUCTOSE. Fasting. Clin.	Fasting. Chromy.	1st. Specimen Clin.	1st. Specimen Chromy.	2nd. Specimen. Clin.	2nd. Specimen. Chromy.
31.	-	-	75	G	50	G	-	-	75	G F+	50	G
32.	-	-	100	G	25	G	-	-	75	G F++	25	G L
33.	-	-	75	G	50	G	-	-	75	F	25	F
34.	25	G	100	G	75	G	50	G++ L	200	G+ F+++	50	G F
35.	50	G+ L	75	G	50	G	50	G	75	G F	50	G F
36.	25	G L	100	G	50	G	25	G L	75	G F++	50	G F
37.	100	G+++ L	200	G++++ L	75	G++ L	75	G++ L	100	G+ L F++	50	G+ L F
38.	25	G L	100	G++ L	25	G L	-	-	100	L F++	50	L Gal. F+
39.	50	G+ L	200	G++++ L	200	G++++ L	25	G+ L	200	G+ L Gal F+	50	G L Gal F
40.	-	-	100	G+++ L	50	G+ L	-	-	100	L Gal F++	50	L Gal F+

present. It follows, therefore, that these signs need not necessarily correspond to those commonly employed in clinical practice to denote concentrations of 25 ---- 200 mg. of sugar per 100 ml. of urine.

The chromatograms showed that the administration of glucose, both to the gravid and non-gravid women, resulted in the excretion of varying quantities of glucose. This was found not only in the first urine specimen which was associated with the period of elevated blood sugar levels, but also in the majority of cases, in the final specimen which was a product of blood sugar values approximating to the fasting levels.

The injection of fructose, however, produced notable qualitative differences in sugar excretion both between gravid and non-gravid, and between the three groups of pregnant women.

When fructose was administered to the 10 non-gravid subjects, fructose alone was excreted and was present in all 10 of the first and nine of the second specimens of urine.

In the pregnant series, the pattern of sugar excretion following the injection of fructose was less predictable, and this was most marked in the final collections of urine.

Normal Pregnancy:- The final specimen yielded fructose in four of the 10 tests. Glucose was present on four occasions.
(Chromatography: Glucose 1, Lactose 3, Glucose with Lactose 2, Glucose with Lactose and Fructose 1, Fructose 3).

Twin Pregnancy:- Fructose was present in all 10 specimens. Glucose was present only once.
(Chromatography: Glucose with Lactose and Fructose 1, Lactose with Fructose 4, Fructose 5).

Glycosuria:- Fructose occurred in eight of the10 specimens. Glucose was present in seven specimens.
(Chromatography: Glucose 1, Glucose with Lactose 1, Glucose with Fructose 3, Glucose with Lactose and Fructose 1, Glucose with Lactose, Fructose and Galactose 1, Fructose 1, Fructose with Galactose and Lactose 2).

Thus, following an intravenous injection

of fructose, it was possible to demonstrate by means of chromatography, distinct qualitative differences in sugar excretion between non-gravid and gravid women.

In the case of the non-gravid, fructose was excreted with conservation of glucose. In the gravid series, twin pregnancy most closely followed this pattern, but the cases of normal pregnancy diverged from it, and this divergence was most pronounced in the group of women having renal glycosuria.

Summary of results.

1. In normal pregnancy, the fasting blood sugar was lower than that of the non-gravid, and the response of the individual to the intravenous administration of glucose resulted in lower total blood sugar values than those similarly obtained in the non-gravid. Despite the inferior blood levels, there was no significant difference in the concentrations of urinary sugar.

2. In cases of twin pregnancy, this depression of blood sugar levels was accentuated in both the fasting and post-injection specimens, but there

was no reduction in urinary sugar concentration from that found in normal pregnancy.

3. The cases of glycosuria showed a similar response to the normal gravid with respect to blood sugar values, but there was a significantly higher concentration of urinary sugar.

4. In all groups, sugar continued to be excreted even after the fasting blood levels had been regained.

5. The fructose load tests produced results parallel to those obtained with glucose in the respective groups, although as had been expected from theoretical considerations, the resultant total blood sugar levels were lower.

6. Qualitatively there was a notable difference in sugar excretion following intravenous fructose, not only between non-gravid and gravid, but also between the three groups of gravid subjects.

COMMENTARY.

These load tests have shown that the intravenous injection of a fixed mass of sugar produced significantly lower total blood sugar levels in gravid than in non-gravid women.

This, however, did not effect a sparing in urinary sugar loss. Indeed, in the cases of twin pregnancy, where post-injection levels were least elevated, there was no difference in the concentrations of urinary sugar from those obtained in the normal pregnancy group.

It is unlikely, therefore, that the depression of the response, as indicated by blood sugar levels, is effected by a homeostatic mechanism having the purpose of conserving carbohydrate in pregnancy.

Although the resultant blood sugar values were essentially the same as those of normal pregnancy, the cases of renal glycosuria excreted higher concentrations of sugar in the urine. In addition, the tendency shown by all the gravid subjects to excrete glucose following fructose administration was most marked in this group.

The excretion of glucose following the administration of fructose might be accounted for in three ways:-

1) Conversion of fructose to glucose.

This would postulate not only that the hepatic

mechanism for converting fructose to glucose is more generally efficient in the gravid than in the non-gravid state, but that it is most advanced in cases of glycosuria and least developed in twin pregnancy.

There is no evidence to warrant such a conclusion.

II) The possibility that fructose passes into storage from the total blood mass more rapidly than glucose.

Judging by the lower curves (figs. 2 and 3) this more rapid absorption of fructose might be true in all cases. If, however, the gravid were preferentially treated in this respect, one would not expect parallelism between the respective curves for glucose and fructose. The relative differences between gravid and non-gravid are the same in both instances.

III) Alterations in renal excretion and re-absorption.

Any differences in the concentration or quality of sugar appearing in the urine in these cases must be due not only to quantitative, but also to qualitative alterations in renal excretion and re-absorption.

In general, pregnancy may therefore be said to affect carbohydrate metabolism in at least three ways:-

a) The blood sugar regulating mechanism is set at a lower level, while at the same time

b) the excretion of sugar is permitted at levels significantly lower than those at which the same concentrations would be excreted by the non-gravid; and

c) by qualitative changes in the excretion and re-absorption of the kidney.

It would seem reasonable to suggest that these effects might result from adaptive changes in the endocrine system.

As is well known, diabetes mellitus may be aggravated by other conditions of hormonal imbalance such as hyperthyroidism. Hypertrophy of the thyroid gland is a common clinical finding in gravid women. Again, it has been shown that glycosuria may be induced, both in the experimental animal and in the human subject, by the exhibition of one of several hormones. Notable among these are the adrenal steroids and the pituitary

adrenocorticotrophic hormone (A.C.T.H.).

Ingle (1941) demonstrated adrenal steroid diabetes in normal rats and using the same experimental animal, Ingle et al. (1946) were able to produce a similar effect using pure adrenocorticotrophic hormone. In the human subject, Browne (1943) and Conn et al. (1948) induced decreased carbohydrate tolerance by treatment with A.C.T.H.

While there is ample evidence that the corticosteroids are greatly increased in pregnancy Venning (1946) ; Parviainen et al. (1950) ; Bayliss et al (1955) ; Schuller (1957) ; Cassano and Tarantino (1957), the effect of pregnancy on the secretion of adrenocorticotrophic and thyrotrophic hormones by the pituitary is still uncertain. Indeed, it appears that in some species (e.g. rat, cat, guinea pig, mouse) hypophysectomy performed during the second half of gestation is compatible with the maintenance of pregnancy, presumably by placental substitution; in other species such as the rabbit, foetal death

and abortion occur, (Selye, 1947).

These hormones, however, which have just been considered are hyperglycaemic agents, and they produce glycosuria by elevating blood sugar levels in excess of the normal physiological range. The increased secretion of these substances is unlikely to account for glycosuria in pregnancy. It has been shown in the present study that following the intravenous administration of sugar, the resultant blood levels in pregnancy - and particularly those of twin pregnancy - are significantly lower than in the non-gravid. If the glycosuria were to be ascribed to the increased cortico-steroids or A.C.T.H., one would expect the blood levels in pregnancy to be higher than those in the non-gravid.

It may be that this seeming paradox of glycosuria in association with relatively low blood sugar values, together with the qualitative changes in renal excretion and re-absorption, could be accounted for by hormonal changes peculiar to the gravid state. Certainly it is possible that

the endocrine status of the maternal organism could be the resultant of an interaction between her own hormones, those of foetal origin and those elaborated by the placenta.

For example, it might be conjectured that foetal insulin contributed to the relatively low maternal blood sugar values. Baird and Farquhar (1962), who acknowledge that it is not known whether unlabelled insulin (either exogenous or endogenous) crosses the placenta, have estimated the plasma insulin activity (P.I.A.) in maternal and cord blood samples taken simultaneously at delivery. Their finding that in normal (as opposed to diabetic) cases, the P.I.A., in cord blood is very much less than in the peripheral venous circulation of the mother, is in agreement with that of Santos et al. (1955). It is unlikely, therefore, that the foetus can make any significant contribution to maternal insulin.

The role of the placenta in the regulation of the maternal carbohydrate metabolism is probably much more active than that played by the foetus. According to Selye (1947), the decidua

and placenta are the only structures, apart from the liver and muscles, in which significant amounts of carbohydrate are stored. Furthermore, this organ is known, either by direct or indirect evidence, to produce various folliculoid hormones, mammogenic hormone, luteotrophin, luteoids and perhaps even testoids. In particular, Selye points out that "the placenta is an extraordinarily rich source of gonadotrophins - especially L.H."

The influence of chorionic gonadotrophin (L.H.) on kidney function has been made the subject of an investigation by the Italian workers Donato and Turchetti (1953). According to these authors, the administration of chorionic gonadotrophin (L.H.) to normal non-gravid women of childbearing age produces a significant reduction in the tubular re-absorption of glucose.

If this claim were correct it might explain the ready tendency to glycosuria, despite the depressed blood level response to administered sugar, which has been shown by the gravid women in this investigation. It would not, however, explain why these blood levels were depressed.

Clearly another factor such as sensitivity to insulin must be concerned.

In view of these considerations, it was decided to investigate the influences of (a) chorionic gonadotrophin and (b) exogenous insulin on blood sugar levels and the urinary output of sugar in the following series of studies.

SECTION III.

THE ASSESSMENT OF CARBOHYDRATE TOLERANCE BY MEANS OF CONTINUOUS INFUSION.

The superiority of the intravenous route for the administration of carbohydrates has been acknowledged in the preceding section of these studies. It must be recognised, however, that there may be a disadvantage inherent in the single injection technique in that a relatively massive dose of carbohydrate is presented to the mechanisms of utilisation and disposal in minimal time. It seemed, therefore, that the tests might be rendered more sensitive if a more physiological situation could be created by presenting the dose over a longer period. Accordingly, it was decided to administer the carbohydrate by continuous infusion in the investigations to be described in this section.

GENERAL METHOD.

The selected cases were given a diet which included 300 g. carbohydrate for three days prior to testing. They were fasted from 10 p.m. on the evening before each test and in the morning

a fasting specimen of urine was obtained. Immediately preceding the test, a catheter was inserted and the bladder drained; the catheter was then secured by means of a spigot. This specimen was discarded.

Standard hospital transfusion equipment was used throughout and 10 per cent dextrose solution was administered from a bottle of 540 ml. capacity. The dextrose run through in priming the giving set was retained and the volume measured. By adding this to the residual dextrose at the end of the experiment, it was possible to calculate the volume of fluid and the quantity of dextrose infused.

On introducing the transfusion needle to a suitable arm vein, a fasting specimen of blood was obtained. The drip was then commenced and the time noted.

The object was to raise the blood sugar by a significant amount. At the same time it was desirable to keep the level within physiological limits and below the normal non-pregnant threshold of 180 mg. per 100 ml. blood.

From experience it was estimated that this would require an infusion of 10-15 grammes of dextrose per hour. This was achieved by regulating the rate of flow to 40 drops per minute. Only very minor adjustments were permissible in the course of the test, sudden acceleration having the effect of momentary loading; retardation, though of less serious consequence, having the opposite effect. While this had the inevitable result of producing some variation in the volumes infused, it did not produce a serious obstacle to analysis of the results.

The infusion was discontinued after 120 minutes, the set being disconnected and the residual dextrose in the bottle measured. Specimens of venous blood were obtained from the arm opposite to that used for the infusion at 30, 60, 90, 120, 150, 180 and 210 minutes from the commencement of the test. These, together with the fasting specimen, were retained for total blood sugar estimation (Nelson, 1944). The catheter was released at 75, 135 and 195 minutes and the volumes of urine measured.

A sample of each specimen including the fasting, was retained and tested for sugar by means of "Clinitest" tablets. Where positive reductions were obtained, chromatograms were run to identify the sugars concerned.

Any modifications to meet the needs of individual investigations are described in detail in the text.

A. The influence of chorionic gonadotrophin on dextrose tolerance.

In 1953 Donato and Turchetti reported that there was a reduction in the renal tubular ability to re-asorb glucose (TmG) following the administration of chorionic gonadotrophin to 10 normal non-gravid women of child-bearing age. In four cases 500 i.u. were given daily for eight days; in six cases 2,500 i.u. were given daily for two days. They concluded that the effect appeared to be proportional to the dose administered. Glomerular filtration rate was not affected.

In view of these findings, it was decided to investigate the action of chorionic gonadotrophin in normal non-gravid women subjected to continuous dextrose infusion in order to establish:

(1) The effect on blood sugar levels.

(2) Whether glycosuria resulted.

(3) Whether the hormone appreciably affected the volume of urine passed during the tests.

Method and Materials.

A series of eight non-gravid women of child-bearing age, in whom there was no evidence of endocrinological abnormality was obtained from gynaecological patients in the Royal Samaritan Hospital for Women.

These cases were submitted to continuous intravenous infusion of 10 per cent dextrose as previously described under "General Method" (page 77).

On completion of this test, the normal diet was resumed. The next day they were given an intramuscular injection of 1,500 i.u. of chorionic gonadotrophin ("Pregnyl" - Organon). This was repeated on the three following days. Thus each patient had four daily injections of 1,500 i.u. chorionic gonadotrophin making a total dosage of 6,000 units.

On the day following the completion of the course of hormone (5 days after the original test) the intravenous infusion of dextrose was repeated.

TABLE 19.

TOTAL BLOOD SUGAR (mg. per 100 ml.).

Patient		Time (minutes). 0	30	60	90	120	150	180	210
SC	C	78	114	115	102	99	65	60	60
	G	74	84	77	76	77	70.	71	72
AH	C	81	106	113	180	173	125	121	75
	G	78	97	99	97	85	72	76	76
JH	C	86	112	105	105	105	79	77	75
	G	74	97	110	110	106	70	52	55
MS	C	75	125	133	131	121	76	56	57
	G	79	108	102	84	78	52	58	64
HM	C	78	89	89	85	80	64	72	79
	G	75	85	82	81	77	62	66	69
H	C	70	106	137	139	124	80	60	57
	G	75	113	135	120	111	66	55	60
FC	C	81	115	125	129	120	83	57	69
	G	81	102	99	92	89	65	69	78
JH	C	83	133	135	123	104	73	57	55
	G	81	108	112	110	102	67	59	63

TABLE 20.

Patient.	Dextrose infused in Grammes. Control (C)	Following 6000 i.u. Gonadotrophin (G).
SC	29.0	30.0
AM	31.0	28.5
JN	26.0	32.7
MS	28.3	30.5
HM	26.8	25.3
M	25.4	30.6
FC	24.2	28.1
JM	28.3	29.0
Total	219.0	234.7
Mean	27.4	29.3

TABLE 21.

MEAN BLOOD SUGAR.

Col. (1). Time (minutes).	(2) Control (C).	(3) Hormone Treated (G).	(4) Difference Control-Hormone.	(5) Remarks.
0	79.000	77.125	+ 1.875	Not Significant.
30	112.500	99.250	+ 13.250	Not Significant. (nearly significant at at P = 0.05).
60	119.000	102.000	+ 17.000	Sig. P < 0.05
90	124.250	96.250	+ 28.000	Sig. P < 0.01
120	115.750	90.625	+ 25.125	Sig. P < 0.01
150	80.625	65.500	+ 15.125	Sig. P < 0.05
180	70.000	63.250	+ 6.750	Not Significant.
210	65.875	69.625	- 3.750	Not Significant.

The results are shown in Tables 19, 22.

It may be observed in Table 20 that there was a slightly higher mean glucose input in the repeat tests following the course of chorionic gonadotrophin. This was fortuitous and simplified statistical analysis:

An analysis of variance in blood sugar levels (Table 21) showed that significant differences occurred (1) between patients ($F=52.0$, $P<0.01$), (2) between times ($F=31.8$, $P<0.01$), (3) between C (control) and G (gonadotrophin) ($F=28.0$, $P<0.01$), and there was also a significant interaction between time and type of treatment ($F=2.17$, $P<0.05$).

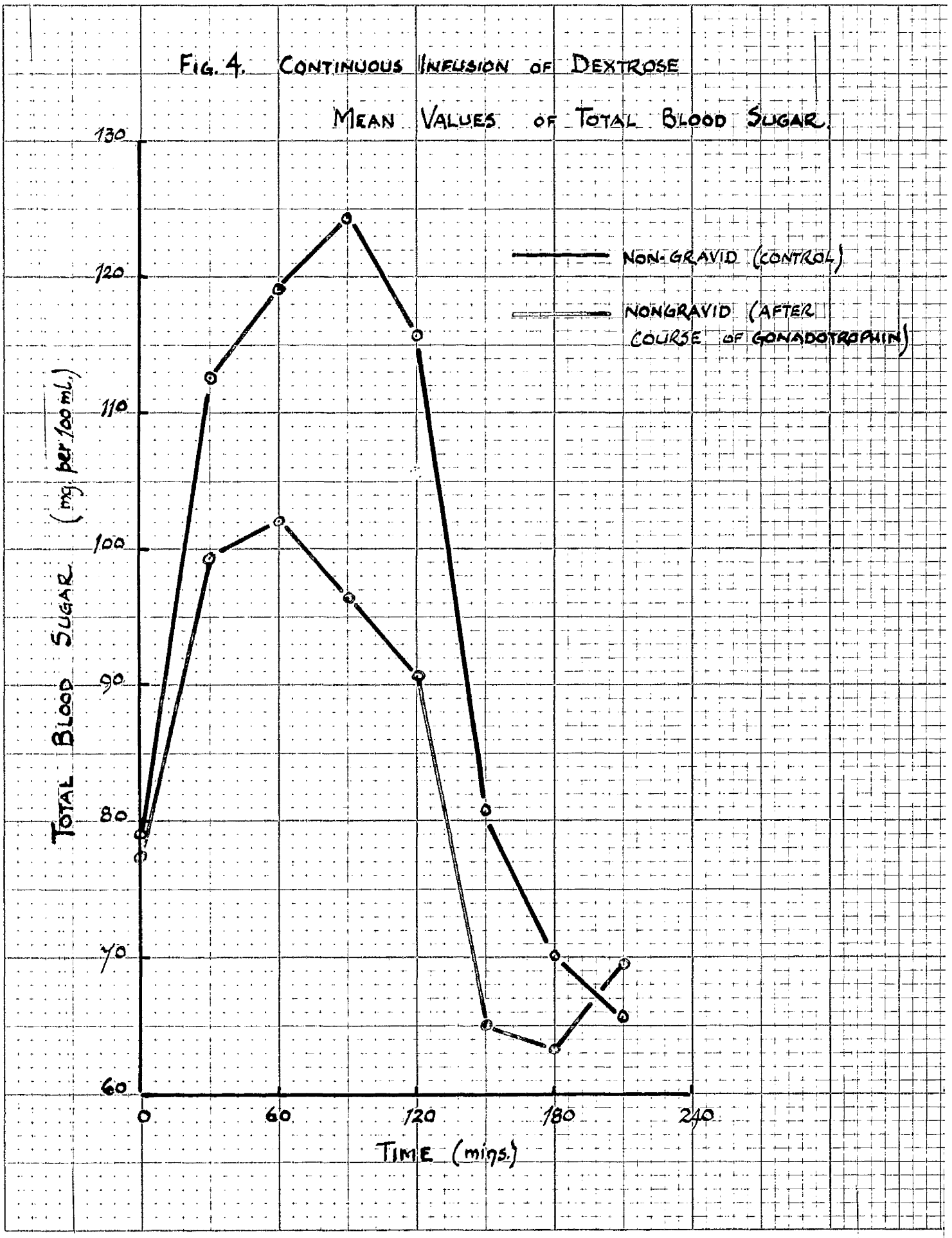
Significant differences between patients were to be expected and require no further discussion.

The differences between times, type of treatment and interaction can best be seen by considering the mean values shown in Table 21 and the corresponding graphs (fig. 4).

The standard error of a single observation is equal to $\sqrt{199.9} = 14.14$ and a difference of

FIG. 4. CONTINUOUS INFUSION OF DEXTROSE

MEAN VALUES OF TOTAL BLOOD SUGAR.



14.0 is required in order to establish a significant difference between any two means (Table 21). Thus considering controls, we find that there is a significant increase between 0 minutes and 30 minutes. Then the mean values for 60, 90 and 120 minutes are at much the same level as for 30 minutes. Thereafter there is a significant fall in blood sugar, until at 210 minutes it has reached a value of 65.875 which is lower, but not significantly lower than the initial value of 79.0

The hormone treated data show a similar trend. The effect of the interaction between time and type of treatment is shown in col.(4). There are no significant differences between controls and hormone treated cases at the start and finish, but there are significant differences in the intermediate stages where the blood sugar after hormone treatment is significantly less than for the control.

The graphs illustrate the same effects.

With regard to urinary output (Table 22), it will be observed that the data are very variable.

TABLE 22.

URINARY OUTPUT (ml.).

Patient.	Type of Treatment.	Time in Minutes.		
		75	135	195
SC	Control.	230	190	180
	Gonadotrophin.	210	170	205
AM	C.	225	100	100
	G.	95	105	55
JN	C.	80	270	265
	G.	395	212	64.
MS	C.	65	90	45
	G.	190	130	55
HM	C.	210	230	150
	G.	210	202	65
M	C.	24	148	82
	G.	65	58	91
FC	C.	62	107	66
	G.	48	70	56
JM	C.	315	225	139
	G.	84	440	57

(The Coefficient of Variation = 53 per cent).

The only significant features are the differences between times as shown by the following mean values:-

Table 23.

Time (minutes).	Mean Values (ml. Urine). Control.	Gonadotrophin.
75	148.9	162.1
135	170.0	173.4
195	128.4	81.0

A difference of 77.5 ml. between any two means is significant. Thus, there is no difference between the three volumes of the control series, but there is a significant fall in the 195 minute mean volume after treatment with gonadotrophin.

Sixty-four urines, including 16 fasting specimens, were obtained from the 16 tests. None yielded a positive reduction for sugar on clinical testing. The absence of sugar was confirmed by paper chromatography.

To summarise these results:-

1) In non-gravid women of child-bearing age, total blood sugar levels were significantly

lowered during continuous intravenous infusion of 10 per cent dextrose following a course of chorionic gonadotrophin. Fasting and post-infusion blood sugar values were not affected.

2) There was no evidence of glycosuria either in the control series or, more especially, after treatment with chorionic gonadotrophin.

3) In the control series of tests there was no appreciable difference in the mean post-infusion volume of urine (195 minutes) from the two specimens obtained at 75 and 135 minutes.

Following the course of gonadotrophin, the post-infusion volume of urine was significantly reduced.

COMMENTARY.

Since the course of gonadotrophin had no effect either on fasting or post-infusion blood sugar values, it seems unlikely that this hormone is, in itself, a hypoglycaemic agent. During the course of the dextrose infusion, however, total blood sugar levels were significantly reduced compared with those in the control series. This suggests that the

gonadotrophin had modified the action of a second substance whose presence was evoked by the raised blood sugar levels during this period. By the same mechanism, when these levels were within normal fasting limits and the stimulus, in consequence, was withdrawn, so the interaction between hormone and hypoglycaemic factor, presumably insulin, fell into abeyance.

While the rôle of oestrogenic hormones as insulin synergists has previously been claimed (Carnot et al. 1928 ; Barnes et al 1933 ; Glen and Eaton 1938) and disputed (Collens et al. 1936 ; Jones and MacGregor 1936) it would appear from the literature that such claims have not been advanced for chorionic gonadotrophin.

These results, which admittedly were obtained by a different method, although employing a slightly higher dosage of chorionic gonadotrophin, appear to be at variance with those of Donato and Turchetti (1953). For example, they found that the hormone reduced the tubular re-absorption of glucose. The results of the present experiments suggest quite the opposite effect, in that chorionic

gonadotrophin assists insulin to reduce blood sugar levels, thereby conserving carbohydrate. Furthermore, the preliminary investigation (Section I) has shown that urinary sugar is minimal during the first 20 weeks of pregnancy when chorionic gonadotrophin is known to have its maximal secretion; therafter, when the secretion of that hormone has declined, renal carbohydrate loss rises to its maximum.

It is unlikely, therefore, that chorionic gonadotrophin is responsible for glycosuria in pregnancy.

In addition, these authors concluded that glomerular filtration rate was unaffected by the course of hormone. It is difficult to reconcile this finding with the present results in view of the significant reduction in urinary output during the post-infusion period. Two explanations for this fluid retention suggest themselves:-

1) If, as Donato and Turchetti suggest, the glomerular filtration rate is unchanged, then

tubular re-absorption of water must be increased during this period. (This cannot be assessed from the data obtained from the present experiments).

2) On discontinuance of the dextrose infusion, the glomerular filtration rate may be reduced in the hormone-treated cases.

There was no significant variation in the three mean volumes of the urine specimens in the control series. In contrast to this, after hormone treatment there was a significant fall in urinary output on withdrawal of the dextrose solution. That is, in the presence of elevated blood sugar and in consequence, endogenous insulin levels, chorionic gonadotrophin did not interfere with urinary output; but when the levels of these two substances mutually fell, gonadotrophin had an inhibitory effect on glomerular filtration.

Although it is well-known that the steroid hormones have the property of producing fluid retention, it is perhaps less generally appreciated that chorionic gonadotrophin, a glyco-protein, may play a similar rôle. There is, in fact,

considerable evidence that the levels of this hormone may be elevated in certain pregnancy states associated with fluid retention and manifest oedema. According to Scott (1958), these conditions may include hydrops foetalis, hydatidiform mole and hydramnios.

Furthermore, it is a well-established observation that pregnancy complicated by diabetes mellitus is frequently associated with oedema. Smith and Smith (1937, 1940)claimed that the gonadotrophin excretion of the pregnant diabetic is relatively high. This claim, subsequently confirmed by Loraine (1949a ; 1949b), is of particular interest in view of the results of the present investigation. These suggest that in addition to promoting fluid retention, chorionic gonadotrophin may take an active part in the regulation of carbohydrate metabolism.

B.

The effects of dextrose infusion on the total blood sugar levels, urinary output and sugar excretion of normal gravid women.

In the investigation which has just been described, it was observed that the administration of chorionic gonadotrophin modified the response of the non-gravid woman to the continuous infusion of dextrose. In particular, the hormone had a depressant effect on total blood sugar levels during the course of infusion. A further effect was a reduction in urinary output during the post-infusion period of the test. Contrary to the experience of Donato and Turchetti (1953), no evidence was obtained to suggest that the gonadotrophin was capable of inducing glycosuria.

Because of these findings, it was decided to examine the effects of a similar infusion in normal gravid women. The objects of this investigation were to determine whether this procedure would produce glycosuria in these subjects; further, to compare the resultant blood sugar levels and urinary volumes with those which had been obtained in the non-gravid series.

TABLE 24.

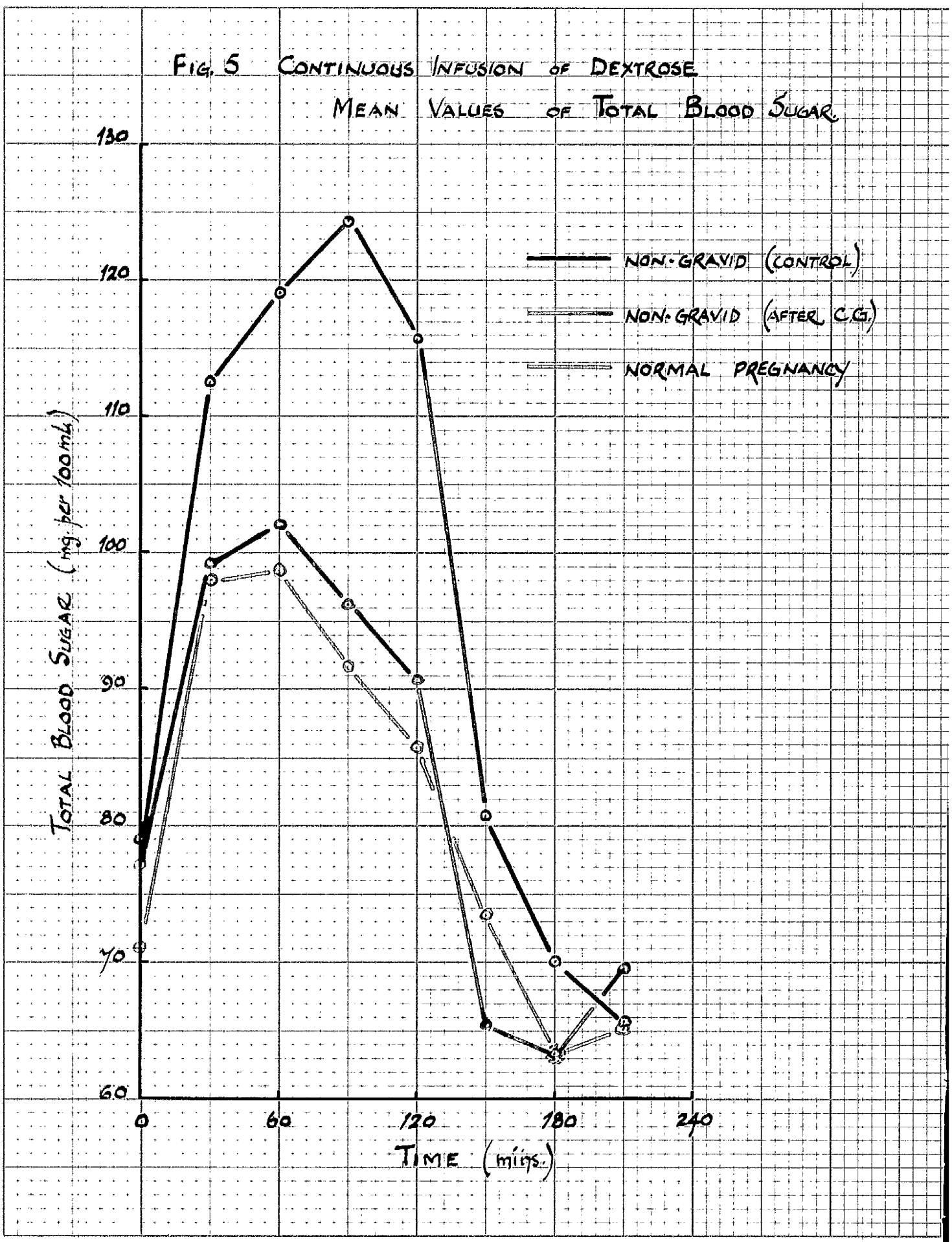
NORMAL PREGNANCY - DEXTROSE INFUSION.

Total Blood Sugar (mg. per 100 ml.).

Case No.	Time (mins.) 0	30	60	90	120	150	180	210	Dextrose infused (Gms).
41.	68	95	100	90	79	68	55	62	32.9
42.	69	87	89	92	88	76	63	65	27.0
43.	66	81	82	79	78	66	57	58	29.2
44.	74	111	106	90	80	72	63	64	31.8
45.	70	87	89	97	90	78	64	68	27.0
46.	78	133	118	96	92	89	78	70	32.6
47.	73	87	95	89	85	65	60	65	27.3
48.	76	104	110	101	94	74	66	70	28.5
Mean	71	98.1	98.6	91.8	85.8	73.5	63.3	65.3	29.5

FIG. 5 CONTINUOUS INFUSION OF DEXTROSE

MEAN VALUES OF TOTAL BLOOD SUGAR.



Method and Materials.

A series of eight normal gravid women was obtained. These subjects, whose pregnancies were over 30 weeks duration, were given a continuous infusion of ten per cent dextrose as previously described under "General Method" (p. 77).

Results. (a) Total Blood Sugar Values.

The resultant total blood sugar values are shown in Table 24 . The mean values are contrasted with those obtained from the non-gravid series, both before and after treatment with gonadotrophin, in fig. 5 . (Advice has been given that the present results approximate so closely to those obtained in the non-gravid after gonadotrophin treatment that no further statistical analysis is necessary).

(b) Urinary Volumes.

The urinary volumes are shown in Table 25 . These results are compared with those previously obtained from the non-gravid subjects in the following analysis:-

TABLE 25.

NORMAL PREGNANCY - DEXTROSE INFUSION.

URINARY VOLUMES (ml).

Case No.	1st. Specimen.	2nd. Specimen.	3rd. Specimen.	Total
41.	28	240	140	408
42.	148	108	63	319
43.	10	45	24	79
44.	140	28	30	198
45.	124	124	150	398
46.	32	12	32	76
47.	74	125	90	289
48.	152	12	23	187
Mean	88.5	86.8	69	2443

Table 26.

Urinary Volumes - Mean Values (ml).

	75 mins.	135 mins.		195 mins.	Overall
Cases 41-48	88.50	86.75	Post-infusion specimen.	69.00	81.42
Non-gravid (SC,AM,JN etc.	148.88	170.00		128.38	149.08

An analysis of variance showed that there was a highly significant difference between the overall mean 81.42 and 149.08 ($F = 14.7$, $P<0.01$).

A difference of 62.7 is required to establish a significant difference between any two means of the above table excluding the overall means.

Thus, although there was no difference between the two groups in the mean values of the individual specimens, the mean total urinary output of the gravid subjects was significantly reduced.

e) Urinary Sugar.

The results for urinary sugar, as determined by "Clinitest" and chromatography, are shown in Table 27. Despite the relatively low blood sugar values, seven of these eight late pregnancy cases excreted sugar in the urine. Lactose was present

TABLE 27.

NORMAL PREGNANCY - DEXTROSE INFUSION.

Urinary Sugar by "Clinitest" (mg. per 100 ml.) and Chromatography.

	Fasting.		1st. Specimen.		2nd. Specimen.		3rd. Specimen.	
Case No.	Clin.	Chromy.	Clin.	Chromy.	Clin.	Chromy.	Clin.	Chromy.
41.	-	-	-	-	-	-	-	-
42.	25	L	25	L	-	-	-	-
43.	25	G L	50	G+ L	50	G+ L	50	G+ L
44.	25	L	25	L	25	L	25	L
45.	25	L	25	L	-	-	-	-
46.	25	L	25	L	25	L	25	L
47.	25	L	50	G+ L	25	G L	25	L
48.	50	G+ L	25	G L	50	G+ L	25	G L

in all of the 29 specimens which yielded a reduction. In ten of these (three cases), glucose was present in addition to lactose, and was the predominant sugar in six of the specimens.

Summary of Results.

1. In normal pregnancy, dextrose infusion produced blood sugar levels closely approximating to those obtained in non-gravid subjects who had been given a course of chorionic gonadotrophin prior to the infusion.

2. The induced glycaemia was arrested in both of those groups at an earlier stage and at lower blood sugar levels than in the control tests performed on the same non-gravid women prior to treatment with chorionic gonadotrophin.

3. In all three groups, blood sugar levels began to fall in the course of the infusion. The maximum levels were substantially below those generally accepted as the upper physiological limits.

A hypoglycaemic "rebound" occurred in all cases.

4. Although the resultant blood sugar levels of the pregnant and gonadotrophin-treated cases were essentially the same, glycosuria resulted only

in members of the former group.

5. The mean total urinary output of the pregnant women was significantly lower than that of the non-gravid.

Commentary.

It may be seen from Tables 19 and 24 and Fig. 5 that the mean total blood sugar values obtained in the pregnancy series closely approximated to those of the non-gravid cases after these subjects had been treated with chorionic gonadotrophin. Not only are the values for both significantly lower than those of the non-gravid controls, but their corresponding graphs display a notable difference in pattern from those of the latter group.

As one might have expected, an immediate glycaemia was induced in all three groups following commencement of the infusion of the dextrose. However, this glycaemia did not continue to increase throughout the entire two hours during which the infusion was being maintained. On the contrary, even although the generally accepted upper physiological limits in the non-gravid were never

attained, the elevation of blood sugar levels was arrested some time before the infusion was discontinued.

In the case of the non-gravid control group, the arrest occurred 30 minutes before this event; but after these subjects had been treated with chorionic gonadotrophin, the maximum glycaemia occurred 60 minutes before discontinuing the infusion. A pattern identical to this was obtained in the pregnancy group.

Thus, in all three groups, blood sugar levels had begun to fall some 30 - 60 minutes before the infusion of dextrose had been discontinued. Thereafter the rate of fall was very much accelerated so that fasting, or indeed sub-fasting values, were reached in the ensuing 30 minutes of the tests.

Post-infusion hypoglycaemic "rebound" is in itself a well-known phenomenon. It is generally accepted that this is effected by endogenous insulin, the secretion of which has been provoked in response to hyperglycaemia. Failure to maintain

the elevation of blood sugar, as may happen for example when dextrose is given by a single injection, will result in the release of excessive insulin in response to this stimulus. In consequence of this excess, a reactionary hypoglycaemic "rebound" may ensue.

Not only has this been illustrated in the results of these infusions, but in all three groups a limited "rebound" had begun even before the infusion was discontinued. In the pregnancy cases and in the non-gravid following treatment with chorionic gonadotrophin, this began earlier and at significantly lower blood sugar levels than those of the non-gravid controls. This finding indicates that in the two former groups, there was a common factor modifying the action of endogenous insulin. That this modifying influence was effective only while the dextrose was being infused, is suggested by the fact that there was no significant difference between their fasting and more remote post-infusion values, and those of the non-gravid controls.

Despite the low blood sugar levels which were obtained, the majority of the subjects in the pregnancy group excreted sugar throughout the test. Most of their specimens of urine contained lactose. The ready tendency to excrete lactose has been a prominent feature of the gravid cases throughout these studies. Indeed it would seem reasonable to conclude that there is virtually no threshold for this sugar. Support for this is given by Thorpe (1947), who states that the disaccharides for their utilisation must be broken down to their simpler component sugars. They cannot be hydrolysed in the body. As a result, if they are absorbed into the blood from the gut (or, presumably, the mammary gland) they are treated as foreign substances and excreted unchanged.

In addition to lactose, glucose was excreted by three of the pregnant subjects. (It may be recalled that sugar was detected in none of the urine specimens of the non-gravid, either before or after treatment with chorionic gonadotrophin).

The blood sugar levels induced by infusion in the normal pregnancy cases closely approximated to those which had been obtained in the gonadotrophin-treated non-gravid subjects. Nevertheless, glucose was excreted by members of the former group only. It is clear, therefore, that this excretion is not merely a resultant of these levels. A more likely explanation lies in the function of the kidney in normal pregnancy.

In this connection it has been found in this investigation that the mean urinary output in the pregnancy series was substantially below that of the non-pregnant cases. There are three ways in which this could have been achieved:-

(1) Reduction in glomerular filtration rate.

(2) Increased tubular re-absorption of water.

(3) By a combination of (1) and (2).

The glomerular filtration rates of normal non-gravid and normal pregnancy cases have been compared by Welsh and Sims (1960). Their finding that a significant increase in glomerular filtration rate occurs in normal pregnancy is in agreement with that of Sims and Krantz (1958).

In the light of these results, the reduction in the overall urinary output noted in the normal pregnancy cases of the present investigation must be ascribed to an increase in the tubular reabsorption of water.

C. The influence of exogenous insulin on the effects of dextrose infusion in normal gravid women.

In the previous investigation it was observed that the continuous infusion of dextrose to normal gravid women had resulted in blood sugar levels which were significantly lower than those which had been obtained in the normal non-gravid. In contrast, when the non-gravid were again tested after a course of chorionic gonadotrophin, the resultant values were so reduced as to be coincident with those of the pregnancy group.

An additional finding was that the pattern of the "curve" had been altered, so that it now conformed to that of the pregnant subjects. Apart from the generally lower contours, the prominent feature was an earlier arrest in the glycaemia induced by the infusion.

It would seem reasonable to suppose that this arrest, and the subsequent fall in blood sugar, had been effected by the release of endogenous insulin in all three groups of subjects. However, this in itself would not explain the differences exhibited by the non-gravid

on the one hand, and the pregnant and hormone-treated non-gravid on the other. Clearly, a common factor had modified the action of the insulin in the latter two series. Despite this modifying influence and the consequent depressant effect on blood sugar, both lactose and glucose were excreted by members of the pregnancy group.

As a result of these findings, it was decided to investigate the effects of exogenous insulin on the pregnant subjects. It seemed possible that by giving insulin coincident with dextrose in the infusion, what has been described as a "negative feed-back" mechanism, might be brought into play. A mechanism analogous to this has been conceived, for example, in the interaction between the ovarian hormones and those of the anterior pituitary (Hamilton et al., 1945). In the present context this would imply that the exogenous insulin would limit the glycaemia occasioned by infusion. This limitation would then, in turn, reduce the stimulus to

endogenous insulin release.

Theoretically, the optimum effects to be achieved by this procedure would be represented graphically by a general flattening and lowering of the blood sugar "curve". One important consequence of this would be the elimination of the hypoglycaemic "rebound". The principal effect, however, might be the prevention of the excretion of lactose and, more especially, glucose in the urine.

It must be made clear in stating these theoretical considerations, that it was never expected that this complete degree of regulation could be achieved in these experiments. Indeed, it was fully appreciated that the balance in such a physiological mechanism, is of too fine a nature to be attained in such an empirical fashion. Nevertheless, it was thought that by giving insulin at the same time as the dextrose, a limited degree of such control might be introduced.

Method and Materials.

The tests were performed on the eight

TABLE 28.

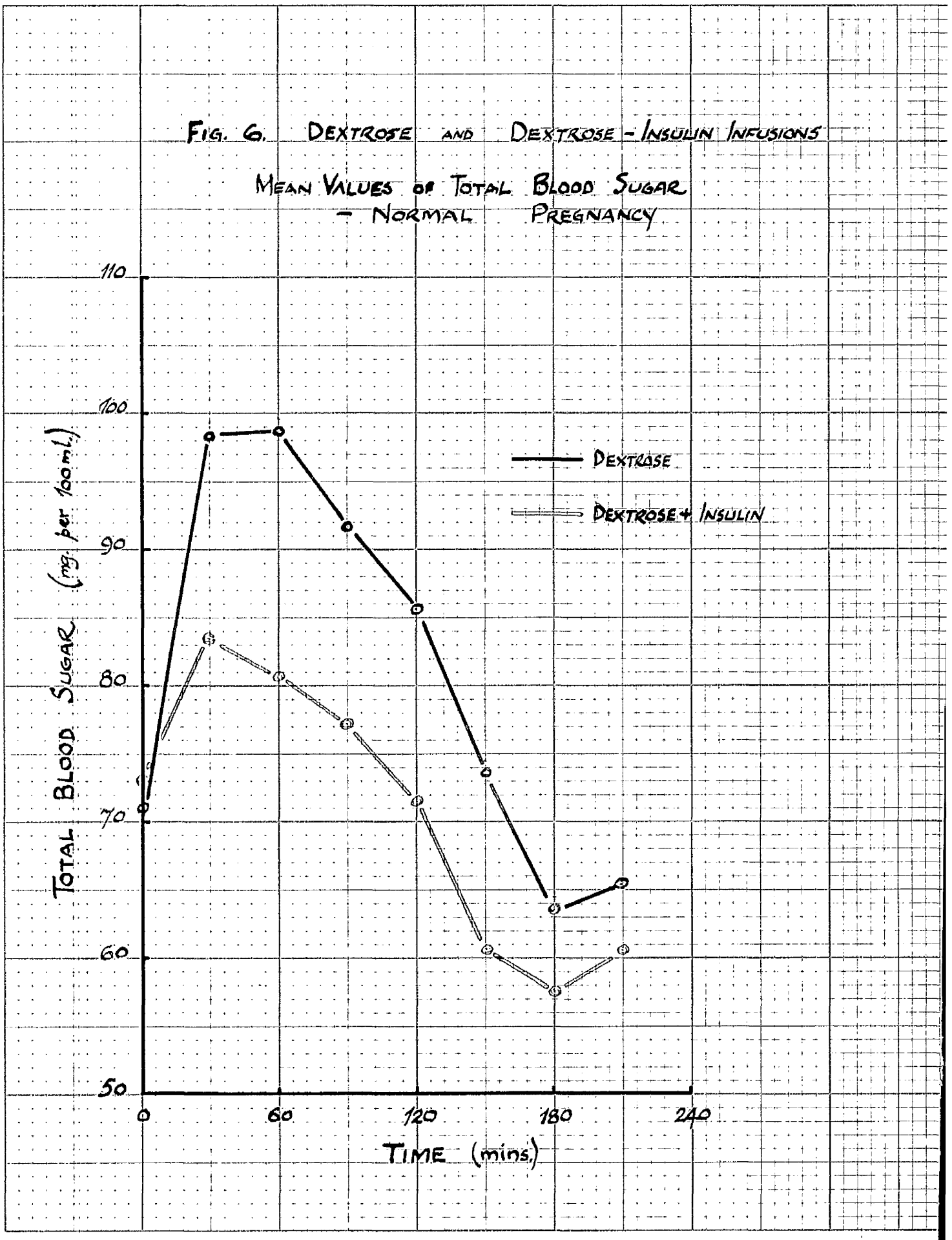
NORMAL PREGNANCY - DEXTROSE-INSULIN INFUSION.

Total Blood Sugar (mg. per 100 ml.)

Case No.	Time (mins.) 0	30	60	90	120	150	180	210	Dextrose Infused (Gms)
41.	75	90	80	76	75	60	60	66	30.9
42.	68	74	79	76	75	55	53	59	33.2
43.	64	76	66	58	57	55	53	54	30.3
44.	78	90	87	85	83	66	66	68	31.9
45.	70	82	79	76	74	55	45	51	29.8
46.	83	98	95	93	85	77	72	68	31.0
47.	70	78	86	80	64	57	53	55	26.0
48.	76	80	74	73	59	58	57	60	27.0
Mean.	73	83.5	80.8	77.1	71.5	60.4	57.4	60.1	30.0

FIG. 6. DEXTROSE AND DEXTROSE - INSULIN INFUSIONS

MEAN VALUES OF TOTAL BLOOD SUGAR - NORMAL PREGNANCY



ante-natal cases who had previously been given a continuous infusion of dextrose. The method was that described under "General Method" (p. 77) except that 20 units (1 ml.) Insulin B.P. were introduced to the bottle of ten per cent dextrose by means of an insulin syringe. The bottle was then inverted several times to ensure distribution of the insulin, (i.e. 1 unit of insulin to every 2.7 g. dextrose).

The influence of the exogenous insulin on (a) total blood sugar values (b) urinary output and (c) excretion of urinary sugar, was then assessed by comparing the results of the present experiment with those which had been obtained when dextrose alone had been administered to the same subjects.

Results.

The results are presented in Tables 28,30 and 32 (cf. Tables 24,25,27) and the mean total blood sugar values resulting from the infusion of dextrose alone, and dextrose with insulin, are contrasted in fig. 6.

(a) Total Blood Sugar Values. The corresponding

TABLE 29.

Ante-natal - Dextrose (D) and Dextrose + Insulin (D + I).

Total Blood Sugar.

Time (mins.)	Mean Values (mg. per 100 ml).		
	D	D + I	Remarks
0	71.750	73.000	Not significant.
30	98.125	83.500	Significant difference
60	98.625	80.750	"
90	91.750	77.125	"
120	85.750	71.500	"
150	73.500	60.375	"
180	64.625	57.375	"
210	65.250	60.125	Not Significant.

mean values obtained with Dextrose alone (D) and Dextrose + Insulin (D+I) are shown in Table 29 from which the following statistical analysis has been prepared :-

An analysis of variance gave the standard error of a single observation of 5.81 ; a difference greater than or equal to 5.8 numerically, will give a significant difference between any two means of Table 29 (on 5% level of significance). The coefficient of variation is 7.7% - a very satisfactory low value. There is a highly significant interaction between the type of infusion and time. This is shown in the table by considering the conclusions that D and D+I do not differ significantly at 0 minutes and 210 minutes, but differ significantly at intermediate times.

Thus, as had been expected, the glycaemia induced by dextrose infusion was significantly less when insulin was added. Contrary to expectation, this did not reduce the subsequent hypoglycaemic reaction. Indeed, the post-infusion hypoglycaemia was accentuated. In short, the addition of insulin

TABLE 30.

NORMAL PREGNANCY - DEXTROSE-INSULIN INFUSION.

Urinary Output (ml.)

Case No.	1st. Specimen.	2nd. Specimen.	3rd. Specimen.	Total.
41.	45	25	15	85
42.	20	128	27	175
43.	30	24	60	114
44.	102	43	23	168
45.	39	50	175	264
46.	62	8	40	110
47.	65	10	20	95
48.	55	32	51	138
Mean.	52.3	40	51.4	143.6

to the dextrose resulted in significantly lower blood sugar levels at all stages, than those which had been produced by dextrose alone.

(b) Urinary Output.

Table 31.

Antenatal. Dextrose (D) and Dextrose with Insulin (D+I).

Urinary Volumes.

Specimen	Mean Values (ml).	
	D	D+I
1st.	88.500	52.250
2nd.	86.750	40.000
3rd.	69.000	51.375

An analysis of variance shows that there is a significant difference between D and D+I ($F = 5.20$, $P<0.05$). If the difference between any two means of the above table is greater than, or equal to, 51 approximately, then it is significant. The coefficient of variation is very high with value 78.8% indicating very variable measures.

The comparison of two means, either at 1st. or 2nd. or 3rd. do not show significance, but with $F = 5.20$, $P<0.05$ there is a significant difference

TABLE 32.

NORMAL PREGNANCY - DEXTROSE-INSULIN INFUSION.

Urinary Sugar by "Clinitest" (mg. per 100 ml.) and Chromatography.

Case No.	Fasting.		1st. Specimen.		2nd. Specimen.		3rd. Specimen.	
	Clin.	Chromy.	Clin.	Chromy.	Clin.	Chromy.	Clin.	Chromy.
41.	25	L	25	L	75	G+ L	25	G L
42.	25	L	25	G L	75	G+ L	25	G L
43.	25	L	25	L	25	L	25	L
44.	50	L	25	L	75	G+ L	25	G L
45.	25	L	50	G+ L	-	-	-	-
46.	25	L	25	L	25	L	25	G L
47.	25.	L	25	L	25	L	25	L
48.	50	L	50	L	25	L	50	G+ L

overall between D and D+I.

Thus, the total urinary output was significantly diminished when insulin was added to the solution of dextrose. At first sight one might ascribe this to a greater conservation of sugar. Certainly it would seem reasonable to suggest that the lowering of blood sugar levels occasioned by the exogenous insulin might have an anti-diuretic effect by reducing osmosis at the level of the kidney. That this explanation is unlikely to be correct, is suggested by the corresponding urinary sugar excretion.

(c) Urinary Excretion of Sugar.

The "Clinitest" and chromatographic results for urinary sugars are shown in Table 32 (cf. Table 27).

It will be seen that, despite the reduction in total blood sugar values, all eight patients excreted sugar in the urine. Excluding the fasting specimens, which were all positive for lactose, 22 of the 24 test specimens contained sugar. Lactose was detected in all of these. Glucose was concurrently present in ten and was in

appreciably higher concentrations (indicated by a + sign in Table 32) than lactose in five of these urines. Six of the eight subjects excreted glucose in the urine when the corresponding blood levels were below the fasting value.

Summary of Results.

(1) In normal pregnant women, the simultaneous infusion of insulin with dextrose, resulted in a significant lowering of the total blood sugar levels at all stages.

The hypoglycaemic "rebound" was not abolished but was accentuated.

(2) Despite the lowering of total blood sugar values, the urinary excretion of glucose and lactose was not eliminated.

(3) The addition of insulin resulted in a significant reduction in the total urinary output compared with that obtained when dextrose alone had been infused.

D. Comparison of the effects of Dextrose-Insulin Infusion in the late pregnancy and puerperal states.

It is generally agreed among clinicians that following parturition there is usually, although not invariably, an immediate and significant fall in the insulin requirements of the diabetic patient. The cause of this reduction is a matter about which there is still considerable uncertainty. One might speculate, for example, that with delivery of the conceptus, the total metabolic requirements are proportionately reduced, thereby reducing the need for insulin. Another explanation for the fall in the insulin requirements of the puerperal subject is suggested by the work of Burt and Kimel (1956).

These authors compared insulin sensitivity in non-gravid, normal pregnancy and early puerperal cases. They found that while sensitivity in the pregnant cases of less than 26 weeks duration was similar to that of the non-gravid, a remarkable decrease developed in the late stages of normal pregnancy. Although their data comparing

ante-natal and puerperal cases afforded less convincing evidence, it suggested that restoration towards normal insulin sensitivity occurred in the early puerperium.

A physiological change of this nature would help to explain the reduction in insulin requirements of the puerperal diabetic. Because of this, it seemed important to substantiate that normal women showed a significant difference in their response to insulin in the puerperal state from that shown by them in late pregnancy.

In the following study, the effects of continuous infusion of dextrose with added insulin were observed on a series of women in the late stages of normal pregnancy. Using the same group, the experiment was repeated after delivery. The results were then compared to determine whether there was any significant difference in their response to insulin as indicated by (a) blood sugar levels and (b) urinary sugar.

Material and Methods.

It will be appreciated that while it is always difficult to obtain consent to perform

TABLE 33.

Ante-natal (a) and Post-natal (b) Dextrose-Insulin Infusion.

Total Blood Sugar (mg. per 100 ml.).

(a)

Case No.	Time (mins.). 0	30	60	90	120	150	180	210	Glucose Infused (Gms.).
49.	71	76	60	50	42	46	50	53	29.7
50.	70	95	99	88	75	66	54	60	32.5
51.	80	93	76	73	67	41	56	69	29.5
52.	75	86	78	70	69	60	65	65	27.0
53.	67	90	69	62	58	65	67	66	25.5
54.	82	99	86	77	67	54	58	64	30.0
Mean	74	89.8	78	70	63	55.3	58.3	62.8	29.0

(b)

49	78	86	66	56	41	39	55	70	28.9
* 50	73	81	66	65	60	33	30	45	32.3
51	73	80	69	48	48	42	50	65	31.5
* 52	73	79	66	54	45	30	34	43	28.8
53	70	75	56	52	50	40	39	49	24.0
54.	73	73	66	54	38	37	45	52	31.3
Mean	73.3	79	64.8	54.8	47	36.8	42.1	54	29.3

* Breast Feeding.

protracted tests, particularly when these must be in duplicate, the difficulties are increased greatly with puerperal cases. Nor is this wholly accounted for by the general resistance in the subjects themselves who quite naturally feel that, with delivery, their object has been attained. Other factors, such as breast feeding and general infant care, tend to militate against the performance of tests during this period.

Nevertheless, a series of six normal antenatal cases agreed to be tested before and after delivery. The infusion of ten per cent dextrose with 20 u. insulin added to the bottle was given in the manner previously described (pp. 110, 112). Using the same patients as their own controls, the tests were repeated on the fourth or fifth day of the puerperium.

Results.

(a) Blood Sugar Values.

The total blood sugar values are shown in Table 33 and their mean values are contrasted in fig. 7. Although the mean

FIG. 7. DEXTROSE - INSULIN INFUSIONS

ANTENATAL AND POSTNATAL MEAN VALUES OF BLOOD SUGAR.

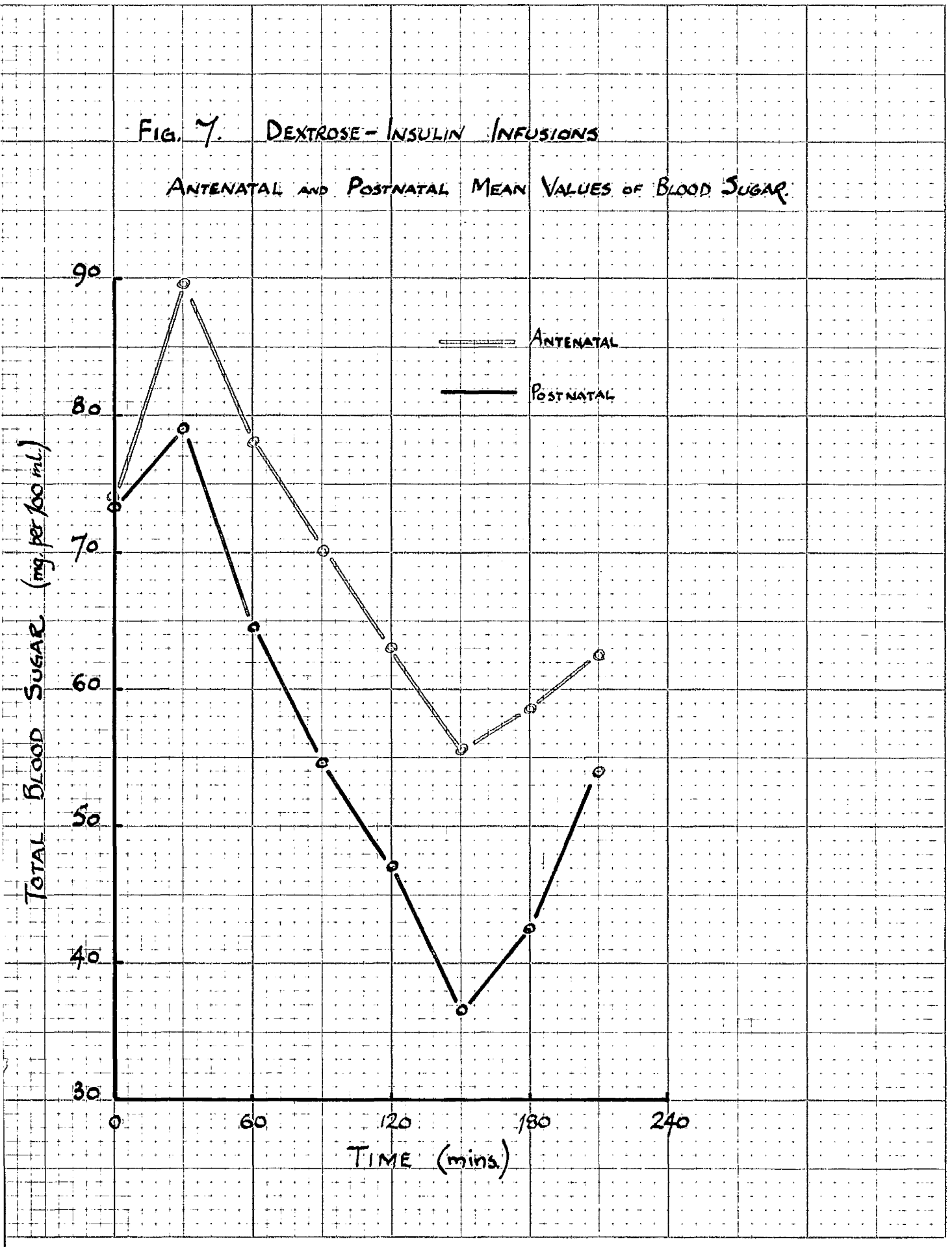


TABLE 34.

Antenatal (A) and Postnatal (P) Dextrose with Insulin.

Total Blood Sugar (mg. per 100 ml.).

Time (mins)	Mean Values		Remarks.
	A.	P.	
0.	74.17	73.33	No significant difference.
30.	89.83	79.00	Significant difference.
60.	78.00	64.83	"
90.	70.00	54.83	"
120.	63.00	47.00	"
150.	55.33	38.00	"
180.	58.33	42.17	"
210	62.83	54.00	No significant difference

dextrose input was essentially the same in the ante-natal period, as that given in the puerperium (i.e. 29.0 g. as opposed to 29.3g.), the blood sugar values for the latter are significantly lower.

An analysis of variance shows significant variations between Ante-Natal (A) and Post-Natal (P) (Table 34) and between times. There is no significance in the interaction between type of treatment and time. The standard error of a single observation = $\sqrt{69.628}$ = 8.344 and a difference of 9.6 numerically is required between any two of the means of the above table for significance (at the 5% level of significance $P<0.05$). The coefficient of variation is 13.3%.

Five of the six cases developed signs and symptoms of hypoglycaemia when they were tested again after delivery. These symptoms, which included profuse sweating, feeling of excessive warmth, weakness and spots before the eyes, all occurred within 20 - 30 minutes of the

TABLE 35.

ANTE-NATAL (a) AND POST-NATAL (b) DEXTROSE - INSULIN INFUSION.

Urinary Volumes (ml.)

(a) Ante-Natal

(b) Post-Natal

Case No.	1st. Spec.	2nd. Spec.	3rd. Spec.	Total	1st. Spec.	2nd. Spec.	3rd. Spec.	Total.
49.	70	15	17	102	42	28	20	90
50.	25	200	130	355	175	145	100	420
51.	34	12	20	66	100	80	43	223
52.	65	40	11	116	55	32	51	138
53.	75	50	60	185	160	80	70	310
54.	25	300	34	359	60	38	30	128
Mean.	49.0	102.8	45.3	197.1	98.7	67.2	52.3	218.2

discontinuation of infusion. This period corresponded to the time of minimal blood sugar values, and in each case the condition corrected itself as these values increased spontaneously.

None of the subjects had developed any of these symptoms when tested before delivery.

(b) Urinary Output.

The urinary volumes excreted ante-natally and post-natally are shown in Table 35 , and the mean values have been analysed:-

Table 36.

Antenatal (A) and Postnatal (P) Dextrose with Insulin Urinary Volumes (ml.)

Specimen	Mean Values A.	P.	Remarks
1st.	49.00	98.67	No significant difference.
2nd.	102.83	67.17	"
3rd.	45.33	52.67	"

An analysis of variance gives the standard error of a single observation, viz. 55.12 with a coefficient of variation of 79.6%. This is very

TABLE 37.

ANTE-NATAL (a) AND POST-NATAL (b) DEXTROSE - INSULIN INFUSION.

Urinary Sugar by "Clinitest"
(mg. per 100 ml.) and Chromatography.

(a)

Case No.	Fasting.		1st. Specimen.		2nd. Specimen.		3rd. Specimen	
49.	25	L	50	G+ L	50	G+ L	25	G L
50.	-	-	-	-	-	-	-	-
51.	25	G	25	L	25	G L	25	G L
52.	-	-	75	G+ L	50	G+ L	25	G L
53.	25	G	50	G	-	-	-	-
54.	25	L	25	L	25	L	25	L
(b)								
49.	50	L	50	G L+	50	L	25	L
* 50.	-	-	-	-	-	-	-	-
51.	100	L	100	L	100	L	100	L
* 52.	-	-	25	L	-	-	-	-
53.	25	L	25	L	25	L	-	-
54.	25	L	50	G L+	75	G++ L	50	L

* Breast feeding.

high, indicating extremely variable material. Little can be expected in the way of significant differences.

(c) Urinary Sugar.

The results of the "Clinitest" and chromatographic investigations of the specimens of urine are shown in Table 37.

In the ante-natal tests five of the cases passed sugar in the urine. Of their 15 specimens, 14 contained lactose. Glucose was also present in eight of these specimens and was the only sugar detected in three others. That is, four of the six patients excreted glucose.

In the post-natal tests, 16 urines (5 cases) contained sugar, lactose being present in all 16 of them. Glucose was also present in 3 of these (2 patients), but in contradistinction to the general ante-natal findings, was the predominant sugar in one specimen only (Case No. 54, Specimen No.2). The concentrations of lactose showed some increase in the puerperium, but this was not as great as had been expected.

It will be seen that one patient (Case No.50) did not pass sugar in any of the urine specimens either ante- or post-natally. (Incidentally, this subject was the only one in the series who did not develop hypoglycaemic symptoms when tested in the puerperium).

Summary of Results.

(1) The dextrose-insulin infusion, when repeated in the puerperium, resulted in total blood sugar levels which were significantly lower than those produced in the same subjects in late pregnancy.

(2) Five of the six puerperal subjects developed symptoms of hypoglycaemia.

None had developed these symptoms when tested in late pregnancy.

(3) It was not possible to establish any difference in urinary output between the late pregnancy and puerperal states.

(4) In the puerperium there was a reduction in the incidence of urinary glucose.

The increase in the concentrations of lactose in the puerperal urines was less than had been expected.

COMMENTARY.

In the opinion of Carrington et al. (1958), (who support the views of Hoet (1954), that placental production of corticotrophin (A.C.T.H) may contribute to reduced carbohydrate tolerance in late pregnancy), tolerance is usually increased promptly after the placenta is delivered. While this may be true, Burt et al. (1957) noted considerable individual difference in recovery rates from the gestational changes which they had detected in carbohydrate metabolism. In many of their patients reversion to the non-pregnant state was incomplete by the fourth or fifth day following delivery. This factor may add to the practical difficulties encountered in the performance of tests on patients during the puerperium.

In addition to those difficulties already mentioned (p. 121), there is therefore the important problem of selecting a favourable time interval following delivery, when tests may be expected to reveal significant physiological changes. At the same time, this interval must be relatively short because the vast majority

of normal cases are dismissed from hospital on the seventh day of the puerperium. Bearing these factors in mind, it was decided that the tests in the present investigation would be performed on the fourth or fifth day after delivery. It was appreciated, however, that a longer interval might well have permitted the establishment of greater differences between the pregnant and puerperal states.

Despite these limitations, it was possible to determine marked differences in the responses to dextrose-insulin infusion, which these cases showed in late pregnancy from those which they later showed in the puerperium. Not only were their blood sugar levels significantly lower when tested after delivery, but this depression was of such a magnitude as to produce clinical evidence of hypoglycaemia in five of the six cases. In contrast to this, neither they nor the eight subjects (cases 41 - 48) who were given a similar infusion in the late ante-natal period, developed symptoms of hypoglycaemia.

Only three puerperal urine specimens

(2 patients) as opposed to eleven ante-natal specimens (4 patients) contained glucose. It is possible that had the interval between delivery and the second test been longer, these differences might have been amplified.

The results of these experiments indicate that there is a substantial increase in insulin sensitivity in the puerperal subject. The work of Burt & Kimel (1956), to which reference has already been made, suggests that this increase may be a relative one following a reduction in sensitivity in late pregnancy. If this were so, then it might be interpreted as a manifestation of recovery to the normal non-gravid status. There is, however, a possible alternative explanation. It might be that the sensitivity to insulin is increased absolutely and is in fact greater in the puerperal than in the non-gravid woman.

Since this possibility had not hitherto been investigated in these studies, it was made the principal subject of the next investigation.

E. Comparison of the insulin sensitivity of normal non-gravid and normal puerperal women.

The principal object of this investigation was to examine the possibility that puerperal women might be more sensitive than the normal non-gravid to the effects of exogenous insulin. For this purpose, a group of normal non-gravid women were given a continuous dextrose-insulin infusion. The effects on total blood sugar levels and on the urinary excretion of sugar were then compared with those which had been observed in the puerperal cases following the same procedure.

A further object was suggested by those results, previously obtained in the late pregnancy subjects, which had indicated that insulin had an anti-diuretic effect. To determine whether this was peculiar to pregnancy, the urinary output of these non-gravid cases was compared with that recorded when dextrose alone had been administered to a similar series of non-gravid women.

Method and Materials.

Six normal non-gravid women were given a dextrose-insulin infusion as previously described (p. 110). The resultant total blood sugar levels and the urinary sugar excretion were then compared with those of the puerperal cases.

To determine whether exogenous insulin had an anti-diuretic effect, the urinary volumes were compared with those obtained from six of the eight non-gravid cases who had been given an infusion of dextrose alone (Section IIIA). These six controls were selected on the basis of the mass of dextrose which each had received, i.e. where this amount most closely approximated to the mean dextrose input (27.3g.) of the present test group. (Although such a close approximation was not altogether necessary, it happened that the mean dextrose input of the controls, so selected, was also 27.3g.).

Results.

(a) Total Blood Sugar Values.

TABLE 38.

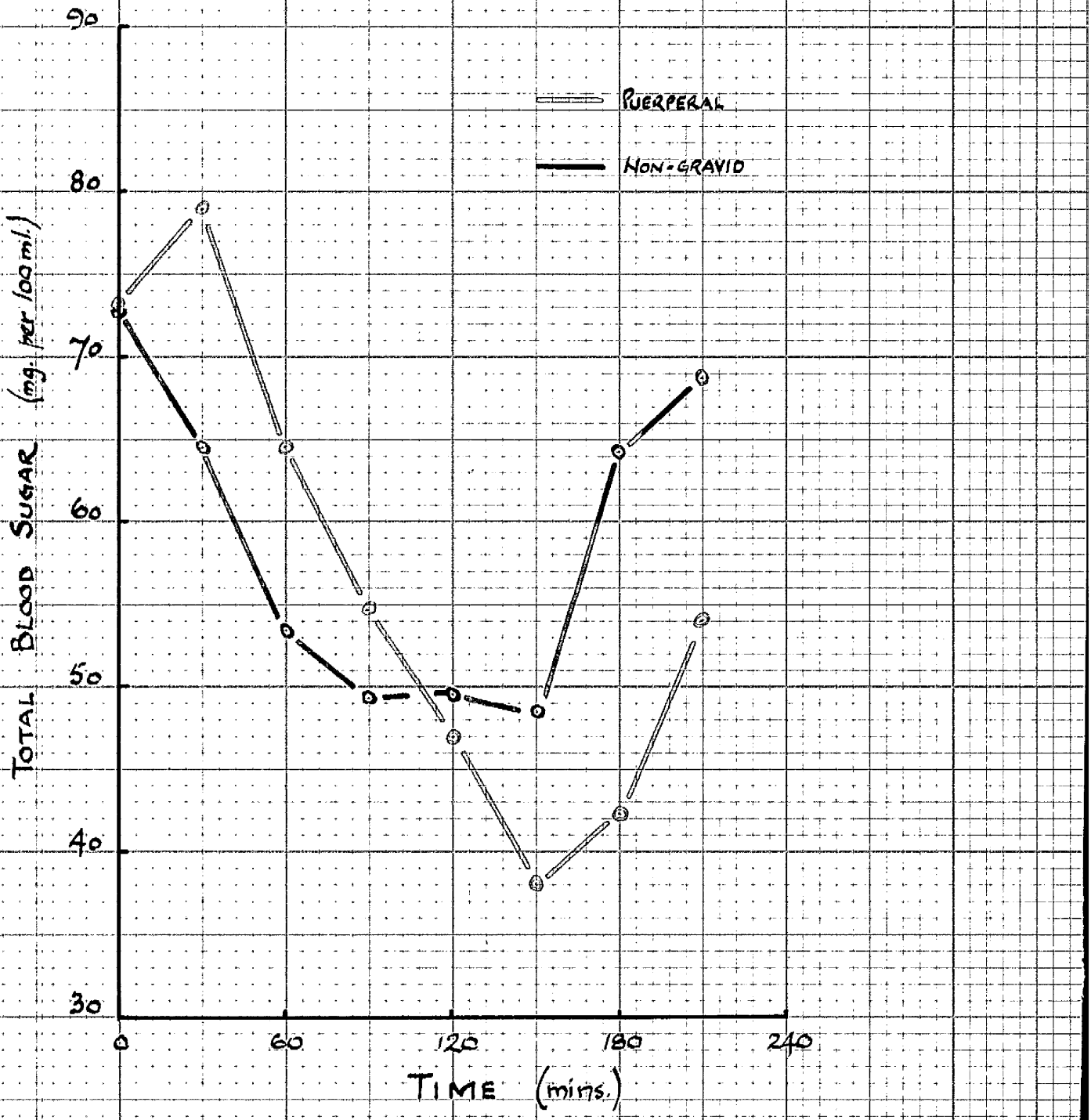
NON-GRAVID - DEXTROSE INSULIN INFUSION.

Total Blood Sugar mg. per 100 ml.

Patient	Time (mins). 0	30	60	90	120	150	180	210
A.	74	77	55	57	61	64	72	74
B.	65	41	20	29	33	49	60	65
C.	77	64	57	48	46	52	65	66
D.	75	39	37	36	43	45	67	76
E.	76	80	69	54	50	36	50	60
F.	70	86	82	71	64	44	71	72
Mean.	72.8	64.5	53.3	49.3	49.5	48.3	64.2	68.8

FIG. 8 DEXTROSE - INSULIN INFUSIONS

PUERPERAL AND NON-GRAVID MEAN VALUES OF BLOOD SUGAR.



The resultant total blood sugar values are shown in Table 38. The mean values are contrasted with those previously obtained in the puerperal subjects (Cases 49-54) in fig. 8 and Table 39.

Table 39.

Non-gravid and Puerperal Dextrose-Insulin Infusions

Mean Values of Total Blood Sugar (mg. per 100 ml.).

	Time (mins.).							
	0	30	60	90	120	150	180	210
Cases A-F	72.8	64.5	53.3	49.2	49.5	48.3	64.2	68.8
Cases 49-54	73.3	79.0	64.8	54.8	47.0	38.0	42.2	54.0

Analysis of these results shows that there is no significant difference at times 0 mins., 30 mins., ---- 210 mins. There is no significant difference between the type of patient.

In short, both sets of patients behave in a similar fashion.

(b) Urinary Sugar.

Sugar was detected in none of the urine specimens.

(c) Urinary Output.

TABLE 40.

URINARY OUTPUT (ml.)

Patient.	Infusion.	Time (mins). 75	135	195	Dextrose Infused (g.)	
A.	Dextrose + Insulin	64	50	25	27.0	
B.	"	82	42	28	26.2	
C.	"	37	50	25	27.2	
D.	"	70	38	38	29.1	Mean = 27.3g.
E.	"	38	226	43	27.5	
F.	"	105	258	200	27.0	
SC.	Dextrose	230	190	180	29.0	
JH.	"	80	270	265	26.0	
MS.	"	65	90	45	28.3	Mean = 27.3g.
HM.	"	210	230	150	26.8	
M.	"	24	148	82	25.4	
JM.	"	315	225	139	28.3	

The urinary output of the non-gravid subjects (A-F), who were given the dextrose-insulin infusion, are shown together with those of the control group (SC --- JM), who had dextrose alone, in Table 40.

The following statistical analysis of these figures has been provided:-

Table 41.

Urinary Output - Mean Values (ml).

	75 mins.	135 mins.	195 mins.	Overall.
Cases A-F	66.0	110.7	59.8	78.83
Controls (SC--JM)	154.0	192.2	143.5	163.23

There is a highly significant difference between Cases A-F and Controls ($F = 18.0$, $P<0.01$). (Excluding the overall means, a difference of 71.9 is required for a significant difference between two means).

Thus, the addition of insulin to the dextrose infusion resulted in a significant reduction in the urinary output of these non-gravid subjects from that of the non-gravid controls who had been given dextrose alone.

These results indicate that the anti-diuretic effect of insulin, previously noted in the late pregnancy cases, is not peculiar to the gravid state.

Summary of Results.

1. There was no significant difference in the total blood sugar levels produced by dextrose-insulin infusion in normal non-gravid women, from those which had been produced in normal puerperal subjects.

2. None of the non-gravid subjects showed evidence of hypoglycaemia, while this had been observed in five of the six puerperal cases.

3. In contrast to the puerperal subjects, none of the non-gravid cases excreted glucose in the urine.

4. The addition of insulin to the dextrose infusion resulted in a significant reduction in urinary output from that of the non-gravid control group who had been given dextrose alone.

Commentary.

In the present study, there was no significant difference in the blood sugar levels induced by

dextrose-insulin infusion in puerperal subjects, from those induced in the non-gravid control group. Nevertheless, the response of the two groups to these levels was quite different. None of the controls showed clinical evidence of hypoglycaemia. In contrast, there was objective as well as subjective evidence of hypoglycaemia in five of the six puerperal subjects.

These results suggest that it may be insufficient to judge insulin sensitivity solely on the degree of depression produced on the blood sugar levels. It is obvious that subjective and more reliably, objective signs must be taken into account in this assessment. When these criteria are applied to the present findings, it must be concluded that the normal puerperal women is more sensitive than the normal non-gravid subject, to the effects of exogenous insulin.

In the previous study (Section IIID), it was confirmed that the normal woman showed an increased sensitivity to insulin when in the

puerperal state, compared with that shown by her in late pregnancy. The results of the present study suggest that this is not merely a partial recovery to the normal pre-gravid sensitivity. On the contrary, they suggest that during these early days following delivery, the sensitivity to insulin in fact is increased above that of the normal non-gravid women. This heightened sensitivity may have a bearing on the decrease in insulin requirements which is commonly shown by the puerperal diabetic.

In the opinion of Skipper (1933) a gain in carbohydrate tolerance often occurs after childbirth, particularly where the diabetes is severe.

This gain was demonstrated in 19 of his 33 cases who got to, or near, term. Insulin reactions, usually occurring in the first few days of the puerperium, were observed in 17 of the 23 women who had been receiving insulin at delivery. Hypoglycaemic coma occurred in three patients; in one of these there had been an accidental

overdosage of insulin. As a result of these personal experiences, Skipper, attributed the maternal death reported by Kaufmann and Kuster (1926) to hypoglycaemia.

The occurrence of hypoglycaemia in the puerperal diabetic has sometimes been ascribed to the effects of lactation. According to this theory, the imbalance is consequent upon glucose being withdrawn from the blood by the breast in the process of milk formation. Against this, Skipper points out that lactation had been suppressed in some of his cases who nevertheless developed hypoglycaemia. This argument is supported by the present findings in normal women. Lactation had been artificially suppressed in four of the five puerperal subjects who exhibited signs of hypoglycaemia.

DISCUSSION AND CONCLUSIONS.

In the Introduction to these studies, reference was made to the diversity of opinion regarding the influence of normal pregnancy on carbohydrate tolerance. The importance of elucidating this problem was stressed not only because of its general physiological importance, but more especially since it might have a considerable bearing on the early recognition and treatment of the pre-diabetic state and of the gravid woman with established diabetes. The need for a further improvement in the management of the pregnant diabetic was indicated by the fact that while the use of insulin had produced a dramatic fall in maternal mortality, there still persisted a significantly high perinatal mortality. While this had been reduced with the evolution of highly skilled obstetric teams, there remained room for considerable improvement. It was thought that some elucidation of possible physiological changes affected by normal pregnancy on carbohydrate tolerance and insulin sensitivity, might help to throw some light on these problems.

As a Preliminary Investigation a series of primigravidae and parous women were observed from early pregnancy until delivery. This was done to determine the influence of age, parity and the stage of gestation on urinary sugar excretion. While it was generally accepted that glucose might be excreted in the urine at practically any stage of normal pregnancy, there appeared from the literature to be some uncertainty as to the stage at which lactosuria might occur. According to some authors its excretion was confined to the puerperium, while it was the opinion of the majority that lactose might on occasion be excreted in very late pregnancy, in addition to the puerperium. Furthermore, there was the possibility that sugars other than glucose or lactose might from time to time contribute to reductions in the clinical testing of pregnancy urines.

To resolve these problems it was necessary to adopt a simple qualitative analytical method capable of the positive identification of individual sugars. The method of paper chromatography

described by Williams (1954) was found to be ideally suited to this purpose, (Appendix A).

In order that the investigation would be concerned with sugar concentrations sufficiently high to be appreciable by clinical methods, the urine specimens were tested first by means of "Clinitest" tablets, and then by Benedict's qualitative solution. Where a positive test was registered with either or both of these, a chromatogram was run for the identification of the sugar(s) contributing to the reduction. A number of the specimens yielding a reduction with Benedict's solution, failed to do so when tested with "Clinitest" tablets. Invariably the absence of sugar in these specimens was confirmed by chromatography. From this it was concluded that these tablets gave a more reliable indication of the presence of sugar.

In this preliminary study it was found that five varieties of sugar were excreted in the urine during normal pregnancy. These sugars, which occurred either individually or in groups of two or three, were lactose, glucose, galactose, fructose

and pentose. The latter two occurred very rarely and were presumed to be from a dietary source.

Lactose was detected as early as the 16th week of gestation. The number of patients having lactosuria showed a progressive increase until term. This finding suggested that the carbohydrate was absorbed into the blood from the breast, as the latter organ underwent development in preparation for lactation. In the present series the concentration of urinary lactose never exceeded 50 mg. per 100 ml.

The excretion of galactose showed a progressive increase parallel to that of lactose, but this sugar was detected less frequently. While the work of Gurin et al., (1941) suggests that it is possible that part of the urinary galactose may be derived from chorionic gonadotrophin, it is more likely that the greater part of this sugar represents a precursor or break-down product of the lactose molecule. Since this molecule cannot be hydrolysed in the body, the only alternative site where this could conceivably happen would be in the urine

itself. It is questionable whether this could occur in fresh or refrigerated specimens. On balance it must therefore be conceded that the bulk of the galactose found in the urine represents a precursor of lactose.

Glucose was detected in the urine specimens of over 75 per cent of the women investigated. The majority of these women began to excrete glucose between the 21st. and 30th weeks of gestation. Most of them continued to do so until delivery. These findings may indicate that the excretion of glucose is related to the completion of placental development and its mature function.

According to Miller et al., (1944), "the presence of glycosuria in the last months of pregnancy in women whose carbohydrate metabolism is otherwise apparently normal, is associated with a foetal and neonatal mortality that is as high as that among the offspring of women with definite signs and symptoms of diabetes". It must be stated that the results of the present investigation have afforded no support for this claim.

While it was found that the stage of gestation

had a marked influence on the excretion of lactose, galactose and glucose, neither age or parity appeared to influence the excretion of sugar. With regard to age, this result probably was to have been expected, since the childbearing period of woman is relatively short to enable significant differences on this account to be determined. However, the failure to find an influence exerted by parity runs contrary to the views of others who have written on this subject. Among these, Labbé and Chevki (1926) claimed not only that the upset in "glyco-regulation" recurred in each pregnancy but also that, to a certain degree, it was accentuated in each successive pregnancy. As has been mentioned in the Introduction, Hoet (1954 ; 1957) expresses a similar opinion. While it is agreed that this may, in fact, occur in some cases the general statement seems much too dogmatic. Certainly it is inconsistent with experience gained during these studies. In the course of these it has been possible to observe a few women with marked glycosuria in one pregnancy, have a subsequent gestation when glycosuria has not

been detected.

In Section II, the carbohydrate tolerance of normal pregnant women was compared with that of the normal non-gravid, cases of twin pregnancy and gravid women who had been pronounced cases of "renal glycosuria" following standard oral glucose tolerance tests. These standard tests had been conducted at the request of several clinicians who had considered that the urinary sugar concentrations registered on routine urinalysis at the ante-natal clinic, had been sufficiently great to require investigation to exclude diabetes. The comparison between normal and twin pregnancy was made because the results of the preliminary investigation had indicated a connection between placental function and the impairment of carbohydrate tolerance. It was considered possible that this impairment might be increased by the larger placental area of the twins.

To effect these comparisons, it was decided to assess tolerance by means of a single intravenous

injection of dextrose. The advantages of the intravenous over the oral route have already been considered in detail (p. 34).

The results of these tests showed that the fasting and more immediate post-infusion total blood sugar levels of the cases of normal pregnancy and renal glycosuria were the same. Those of the normal non-gravid were significantly higher, while those of the cases of twin pregnancy were significantly lower. Despite the differentials in the blood sugar levels induced by the injection of dextrose in the non-gravid, normal pregnancy and cases of twins, the urinary sugar concentrations were the same. In contrast, the cases of renal glycosuria excreted significantly higher concentrations than all of the other subjects, although the corresponding blood levels were the same as those of the normal pregnancy group.

Confirmation of these findings was obtained from the results of the fructose load test which was performed in addition to the intravenous dextrose tolerance test on each of the subjects. When fructose was administered intravenously to the

four groups, the resultant total blood sugar levels, although as had been expected lower than those obtained with dextrose, were parallel to the latter. Thus the differences between the four groups were again established. In addition, it was observed that whereas the non-gravid selectively excreted fructose, the pregnant tended to excrete glucose in addition. This was most marked in the cases of renal glycosuria suggesting not only that the impairment in these cases lay at the level of the kidney, but that this impairment was qualitative as well as quantitative.

It was observed in the course of these load tests that a considerable number of the subjects continued to excrete sugar - and particularly glucose - even after the blood levels had reached fasting or even sub-fasting values. One has made the same observation frequently in the course of the standard oral glucose tolerance tests on gravid patients.

These findings suggest that the conception of a fixed renal threshold for glucose may be

misleading. In this connection, Johns (1930) concluded from a study of 1,100 glucose tolerance tests that there was no such thing as a normal threshold, and expressed the opinion that for all individuals there is an individual threshold. Campbell et al., (1932) went further. These authors believed that the threshold varies in the same person and concluded from their experiments, "it seems that something more than a mere lowered threshold is present in renal glycosuria".

A possible resolution of this problem has been suggested by Govaerts and Lambert (1949). In the view of these authors, it is necessary to define a minimal and a maximal threshold in order to understand the laws governing the excretion of glucose in the presence of a moderate hyperglycaemia. This, they claim, is due to the fact that the nephrons being structurally unequal in size and surface area, are unequal in their reabsorptive capacity. Hence they recognise the maximal threshold as the level of hyperglycaemia at which enough glucose

is filtered to saturate the nephrons with the greatest reabsorptive power. It follows that the minimal threshold is the level of hyperglycaemia at which enough glucose is filtered to saturate the nephrons with the smallest reabsorptive capacity.

Thus Govaerts and Lambert ascribe the occurrence of glycosuria in the presence of normal, or nearly normal, blood sugar levels to a lowering of the minimal threshold. However, they observed in the course of their experiments that the reabsorption of phosphorus and amino-acids was not affected, although these processes are known to occur at the same level of the proximal tubule at which glucose is reabsorbed. As a result, they concluded that the cause of glycosuria in association with relatively low blood sugar levels was likely to be a disturbance in those enzymatic processes which make possible the reabsorption and transfer of glucose through the cells.

To relate this theory in its entirety to the present findings, one would have to conceive of

the cases of renal glycosuria having a greater proportion of smaller, and therefore more readily exhaustible nephrons, than the normal pregnant women. Similarly, in general, those of the non-gravid would have to be larger, while those of the cases of twin pregnancy would require to be smaller than the nephrons of the normal gravid.

While the evidence is thus against a morphological explanation for the existence of a minimal threshold, it in no way precludes the possibility of a partial saturation of the enzymatic processes responsible for the reabsorption of glucose.

According to Homer Smith (1951), glucuresis in glomerular forms is attributable to incomplete tubular reabsorption under circumstances in which the load of filtered glucose exceeds the maximal reabsorptive capacity of the tubules. A partial saturation of these processes might well create such circumstances.

Some support for this is given by the results of the fructose load tests. It is interesting to

compare these results with those of Hansen et al., (1943). These authors found that in the dog a considerable fructosuria is not accompanied by an increased excretion of glucose. They concluded, therefore that in so far as the two hexoses share a common reabsorptive system, it is not that component which determines the maximal rate of reabsorption of either one. In the present investigations it was found that like the dog, the non-gravid subjects did not respond to the fructose load by excreting glucose. In contrast to this, a number of the gravid cases did so and this excretion of glucose was most marked in the cases of renal glycosuria. These findings may indicate that, in the concept of Gammeltoft and Kjerulf-Jensen (1943), adenosine triphosphate was at a more severe premium in these cases.

The relative deficiency in this energy system could well be due to partial saturation by some substance other than the common hexoses. The nature of this substance can only be a matter for conjecture. However, it obviously must be produced

in large quantities during pregnancy, and must also be reabsorbed in the first part of the nephron.

It is possible, therefore, that as a result of the intravenous loads of sugar, a degree of saturation of the renal tubular mechanism for the reabsorption of glucose occurred in all groups, both gravid and non-gravid. In general, the reabsorption was most impaired in the pregnancy cases, not only because of their tendency to excrete glucose in both the dextrose and the fructose tests, but also in view of the significantly lower total blood sugar levels at which this excretion occurred. In particular, the cases of renal glycosuria exhibited most evidence of this disorder. It is also conceivable that this saturation, persisting from the initial stages of moderate hyperglycaemia, could give rise to the subsequent paradox of glycosuria in the presence of fasting and sub-fasting blood sugar values. This has been noted frequently during the present investigations and has been observed

in clinical practice in the treatment of hyperemesis gravidarum and cases of prolonged labour with ketosis. In addition, this concept would explain the failure of exogenous insulin to abolish glycosuria in late pregnancy cases where it had nevertheless effected a significant reduction in their total blood sugar values (Section IIIC).

It has been suggested by Bergqvist (1954) that this tendency of pregnant women to excrete glucose might be due to excess secretion of A.C.T.H. interfering with renal reabsorption. Certainly there is no evidence afforded by the present data to suggest that such a cause could be responsible. One would expect the pregnant cases to show a hyperglycaemia rather than a significant lowering of blood sugar values compared with the non-gravid if this were the case.

A possible solution to the differentials in the total blood sugar levels resulting from the sugar loads has been suggested by the results obtained from the experiments described in

Section III. In this section of the studies, the dextrose was administered by continuous infusion. It was considered that by spreading the load over a longer period, a more sensitive index of the response could thus be obtained by reducing the amount of sugar presented in unit time. In the event, this method proved quite satisfactory. It must be remarked, however, that there is one possible limitation to its use as a routine test for individual patients. This lies in the difficulty of regulating the infusion so that a fixed dose of dextrose is given uniformly in a prescribed interval of time. It is true that in the case of the insulin component of the dextrose-insulin infusion these variations may be of much less importance. This is due to the fact that the duration of action of insulin is not proportional to the size of the dose but is, in fact, a simple function of the logarithm of the dose (Best and Taylor, 1950). However, in the present investigations these objections were overcome by the method of using

groups, or block samples, of subjects. As a result, it was possible, by statistical analysis, to take account of individual variations in the amounts infused.

In the initial investigations described in Section III, it was found that the glycaemia induced by dextrose infusion in non-gravid women was markedly reduced after they had been given a course of chorionic gonadotrophin. Indeed, following this course of hormone, there was no significant difference in the resultant total blood sugar values from those induced by similar infusion in the normal gravid subject. At the same time, glycosuria occurred in members of the pregnant group only. These findings indicate that while chorionic gonadotrophin is not a causal factor in the promotion of glycosuria, it may take an active part in the regulation of carbohydrate metabolism. Furthermore, they may help to explain the different responses in total blood sugar levels which were noted in the normal non-gravid on the one hand, and in the pregnancy

cases on the other, following the intravenous load tests described in Section II. The depressed response observed in the pregnancy groups in general, and in the cases of twin pregnancy in particular, could conceivably have been effected by the corresponding levels of placental gonadotrophin.

While one has been unable to find any reference in the literature to suggest that chorionic gonadotrophin has been investigated in this particular relationship to the regulation of carbohydrate metabolism, it would appear that the effects of other sex hormones have been studied extensively. However, the results of these studies are very conflicting and it is difficult to make a final assessment. For example, there have been controversial reports regarding the effects of oestrogens on carbohydrate metabolism. In this connection, a number of authors have produced evidence that the glycosuria of either experimental or clinical diabetes mellitus can be reduced by oestrogens (Barnes et al., 1933 ;

Nelson and Overholser, 1936 ; Mazer et al., 1935 ; Gessler et al., 1939 ; Spiegelman, 1940). This, however, has been questioned (Collip et al., 1937).

Working on the experimental animal Ingle (1941b) reported that the diabetic state of the partially depancreatized rat can be intensified by the administration of stilboestrol. Mutually supporting results to these were reported by Dolin et al., (1941) who used partially depancreatized ferrets. At the same time, these authors obtained completely negative results using progesterone.

In contrast, Cantilo (1941) reported that large doses of oestrogen and progesterone had effected the diabetic control of his entire series of 40 menopausal and postmenopausal women. In these, insulin treatment and diet had been relatively ineffectual. He ascribed these successes to the inhibiting influence of these hormones on the anterior pituitary.

With regard to the inhibition of the pituitary, reference has already been made

to the work of Glen and Eaton (1938) in the commentary to Section IIIA. These authors reported a case in which insulin produced a greater degree of carbohydrate intolerance and glycosuria than glucose alone. They cite a similar case which was reported by Falta (1924) and refer to the finding of Barnes et al., (1933) that amniotonin (ketohydroxyoestrin) diminished glycosuria in depancreatized dogs. This finding, with that of Tuchman (1937), that the injection of ketohydroxyoestrin diminishes the hypertrophied pituitary glands of castrated animals, suggested to Glen and Eaton that excess pituitary secretion might be responsible for their patient's unexpected reaction to insulin. They therefore considered it rational that ovarian extract might be expected to have a synergic action with insulin. In the event, when the patient was given dihydroxyoestrin in addition to glucose and insulin a greater reduction occurred in the blood sugar levels. Similar success with oestrogens was reported by Carnot et al., (1928) and Rathery and Rudolph (1928).

The latter two authors suggested that for each dose of insulin, there was an optimal dose of oestrogen, and tentatively suggested that an equilibrium between these hormones may be necessary for normal carbohydrate metabolism.

While it must be appreciated that there remains a considerable conflict of opinion regarding the influence of oestrogens on carbohydrate tolerance, these successes are of particular interest in view of the results obtained by White (1947 ; 1959) in the management of the pregnant diabetic. As is well known, this management is based on the theory elaborated by Smith and Smith (1947) to account for the raised levels of serum chorionic gonadotrophin in association with a reduction in serum oestrogens and urinary pregnanediol which they had found to be present in pregnant diabetics. The theory postulates that while, under normal circumstances, the chorionic gonadotrophin is utilized by the placenta in the formation of oestrogen and progesterone, in pregnant diabetics, this mechanism is deranged. Accordingly,

White gives her diabetic patients a dose of stilboestrol and progesterone based on the severity of the disease. While it is generally agreed that her foetal survival rate of 90 per cent is quite remarkable, the actual cause of this salvage is much disputed. Certainly the results of other workers who have employed hormone replacement therapy have been less encouraging (Given et al., 1950 ; Rolland 1954). Thus, many are inclined to the view that the excellent results obtained by White are due to the care taken by the obstetric team in general, rather than to hormone therapy in particular (Lewis, 1956). However, one cannot but feel that a weakness in this argument of the sceptics is the widely recognised fact that, even where the general care is of the highest order, the foetal loss is still considerably higher than the 10 per cent reported by White (Introduction).

It is possible that the working hypothesis used by Smith and Smith to explain their findings may, in fact, be quite erroneous. It might be

that the exhibition of oestrogen and progesterone is effective in these cases as the result of a synergism with endogenous insulin. Furthermore it has been found in these studies that chorionic gonadotrophin appears to play an active part in carbohydrate metabolism in the presence of hyperglycaemia. It is just possible that the elevated levels of this hormone found by the Smiths in the pregnant diabetic may also have exerted some controlling influence on the associated hyperglycaemia.

Since it was considered possible that physiological changes peculiar to pregnancy might affect the response of the established diabetic to insulin therapy, the influence of insulin on normal late pregnancy and puerperal cases was investigated (Section IIIC,D). It was confirmed that the subjects showed a significant increase in their sensitivity to insulin when in the puerperal state compared with the late stages of normal pregnancy. This finding was not unexpected, having been indicated by Burt and

Kimel (1956). Nevertheless there remained unresolved the problem of whether the sensitivity of the early puerperal subject was part of a graduated return to that of the normal non-gravid state or whether she was, in fact, more sensitive to the effects of exogenous insulin than the non-gravid. This matter was investigated in the final study (Section IIIE).

The results obtained from this study indicated that, in terms of the blood sugar levels induced, there was no significant difference in the response of early puerperal subjects from that of the normal non-gravid. At the same time five of the six puerperal subjects exhibited signs of hypoglycaemia. These were entirely absent in the non-gravid group. It would seem reasonable that such clinical evidence should be taken into account in the assessment of insulin sensitivity. Therefore it must be concluded that the normal puerperal subject has a greater sensitivity to the effects of exogenous insulin than the normal non-gravid subject. Furthermore, since lactation had been artificially

suppressed in four of the five cases who showed this evidence of hypoglycaemia, it is clear that diversion of blood glucose for the purposes of lactogenesis is not a major contributing factor in the production of these symptoms.

In Section IIIC it was found that, compared with the volumes of urine passed when dextrose had been given alone, a significant reduction occurred in the urinary output of the late pregnancy subjects when insulin was added to the dextrose infusion. Indeed this reduction was more striking than that which had been observed in the post-infusion volumes of the non-gravid cases after they had been given a course of chorionic gonadotrophin. It was considered possible at that time that the raised blood sugar and, incidentally, endogenous insulin levels, had in some way counteracted the anti-diuretic effect of the gonadotrophin (Commentary, Section IIIA). Thus it was inferred that as a result of this possible interaction the anti-diuretic effect of the gonadotrophin had become manifest only

when the blood sugar and endogenous insulin levels had fallen on discontinuance of the infusion. This subsequent finding that the simultaneous administration of insulin with dextrose produces a marked fall in urinary output indicates that the assumption of an antagonism of this kind between insulin and the gonadotrophin was quite wrong.

While no satisfactory explanation can be given at present for this unexpected finding, it can be concluded quite safely that the anti-diuretic effect of insulin is not attributable to an interplay between this hormone and those peculiar to pregnancy. The results obtained in Section IIIE clearly demonstrate that insulin has a similar restrictive effect on the urinary output of the non-gravid.

From the results of these studies the following conclusions would appear to be justified:-

(1) A variety of sugars including lactose, glucose, galactose and more rarely, fructose and pentose may be detected in the urine in the course of

normal pregnancy.

These may occur either individually or in groups of two or three.

(2) From early pregnancy, there is a progressive increase in the incidence of lactosuria.

(3) There is also a progressive increase in the incidence of the excretion of galactose but this sugar occurs much less frequently than lactose.

(4) Over 75 per cent of women excrete glucose in the course of normal pregnancy. This appears to be quite independent of either age or parity.

While some women may excrete glucose in early pregnancy, the majority begin to do so between the 21st. and 30th weeks of gestation. In most of these, this continues until delivery.

(5) The fasting blood sugar level of the normal non-gravid is higher than that of the normal pregnancy subject, while that of twin pregnancy is lower.

These differences are maintained in the response of the three groups to the intravenous administration of a fixed load of sugar.

Nevertheless the resultant concentrations of urinary sugar are essentially the same.

(6) In contrast to the above, pregnant cases of "renal glycosuria" while having the same fasting blood levels and showing the same response to the sugar load as normal pregnant subjects, pass significantly higher concentrations of sugar in the urine.

(7) The conception of a fixed threshold may be misleading, since all groups may continue to excrete sugar at fasting or sub-fasting blood sugar levels.

(8) Following a course of chorionic gonadotrophin, there is a significant reduction in the glycaemia induced by dextrose infusion in normal non-gravid women.

It is to be noted that the concentration of chorionic gonadotrophin is frequently much increased in pregnant diabetics. During a glucose tolerance test, the increase may have the effect of holding the blood sugar at a lower level than might be found in the non-pregnant states. This could be misleading

in a mild case of diabetes. Furthermore, it could explain those equivocal results obtained from glucose tolerance tests performed on subjects subsequently proved to be in the prediabetic state.

A secondary effect of the chorionic gonadotrophin is a reduction in the urinary output in the period immediately following the discontinuance of the infusion.

(9) In the normal late pregnancy cases, the addition of insulin to dextrose infusion, while producing a significant reduction in the resultant levels of blood sugar, does not abolish the urinary loss of either lactose or glucose.

Thus the employment of urinalysis as an index of the response of the pregnant diabetic to insulin therapy may be dangerous, and could lead to complications from hypoglycaemic episodes.

(10) Normal women, when in the early puerperal state, show an increased sensitivity to the effects of exogenous insulin compared with that shown by them in late pregnancy.

(11) When the same amount of dextrose-insulin solution is administered to early puerperal and normal non-gravid women, the resultant total blood sugar values are the same in both groups.

Nevertheless, the finding that symptoms of hypoglycaemia occur only in the former group at these levels, indicates that the sensitivity of the normal puerperal subject is greater than that of the normal non-gravid woman.

On the basis of the above, the dose of insulin has to be readjusted very carefully in the puerperium if hypoglycaemic crises are to be avoided. (12) The addition of insulin to dextrose infusion results in a significant reduction in the overall urinary output of both normal non-gravid and normal pregnant women.

Since the glycosuria in pregnant patients was not abolished by insulin, this result cannot be ascribed to reduction in the osmotic effect of urinary sugar. It would therefore appear that this is a specific effect of insulin on kidney function.

BIBLIOGRAPHY.

ALLEN, E. (1939) : Am. J. Obstet. Gynaec, 38, 982.

ANDERSON, W.A.D. (1948) : Pathology, London, KIMPTON, p.635.

BAIRD, J.D. and FARQUHAR, J.W. (1962) : Lancet 1, 71.

BARNES, B.O., REGAN, J.F. and NELSON, W.O. (1933) : J. Amer. Med. Assoc. 101, 926.

BAYLISS, R.I.S., BROWNE, J.C. McC., ROUND, B.P. and STEINBECK, A.W. (1955) : Lancet 1, 62.

BERGER, H. (1952) : J. Amer. Med. Assoc. 148, 364.

BERGQVIST, N. (1954) : Acta. Endocrin. 15, 166.

BERTRAND, N.R. and GILBERT, J.A.L. (1960) : Canad. Med. Ass. J. 83, 753.

BEST, C.H. and TAYLOR, N.B. (1950) : The Physiological Basis of Medical Practice 5th Ed., Baltimore, Williams and Wilkins, p. 672.

BROWNE, J.S.L. (1943) : Josiah Macy Jr. Foundation Conference, cited by Best and Taylor, (1950).

BURT, R.L. and KIMEL, C.A. (1956) : Obstet. and Gynaec. 7, 658.

BURT, R.L., WINSTON-SALEM, N.C. and JULIAN, N. (1957) : Am. J. Obstet. & Gynaec. 74, 551.

CAMPBELL, R.A., OSGOOD, E.E. and HASKINS, H.D. (1932) : Arch. Int. Med. 50, 952.

CANTILO, E. (1941) : Endocrinology 28, 20.

CAPPELL, D.F. (1958) : Muir's Textbook of Pathology, 7th. Ed., London, Arnold, p. 635.

CARNOT, TERRIS and CAROLI (1928) : Bull. et mém Soc. méd. hôp. de Paris 52, 738.

CARRINGTON, E.R., REARDON, H.S. and SCHUMAN, C.R. (1958) : J. Amer. Med. Assoc. 166, 245.

CASSANO, F. and TARANTINO, C. (1957) : Riv. ostet. ǵin., Fir. 12, 471.

COLLENS, W.S., SLO-BODKIN, S.G., ROSENBLIETT, S and BOAS, L.C. (1936) : J. Amer. Med Assoc. 106, 678.

COLLIP, J.B., SELYE, H. and NEUFELD, A. (1937) : Am. J. Physiol. 119, 289.

CONN, J.W., LOUIS, L.H. and JOHNSTON, M.W. (1948) : Proc. Am. Diabetes Assoc. 8, 213.

CORI, C.F. (1925) : J. Biol. Chem. 66, 691.

CRAWFORD, T. (1938) : Arch. Dis. Childh., 13, 69.

DOLIN, G., SAMUEL, J. and GAUNT, R. (1941) : Endocrinology, 28, 840.

DONALD, I. (1959) : Practical Obstetric Problems, 2nd, Ed. London, Lloyd-Luke, p. 84.

DONATO, L. and TURCHETTI, G. (1953) : Boll. Soc. Ital. Biol. Sper., 29, 1166.

DUNCAN, L.J.P. (1956a) : Quart. J. Exp. Physiol., 41, 85.

- - (1956b) : Ibid , p.453.

FAJANS, S.S. and CONN, J.W. (1954) : Diabetes 3, 296.

FALTA, W. (1924) : Klin. Wchnschr. 13, 1315, cited by Glen and Eaton (1938).

GAMMELTOFT, A. and KJERULF-JENSEN, K. (1943) : Acta physiol. scandinav. 6, 368.

GESSLER, C.J., HALSTED, J.A. and STETSON, R.P. (1939) : J. Clin. Invest., 18, 715.

GILBERT, J.A.L. and DUNLOP, D.M. (1949) : Brit. Med. J., 1, 48.

GIVEN, W.P., DOUGLAS, R.G. and TOLSTOI, L. (1950) : Amer. J. Obstet. Gynaec. 59, 729.

GLEN, A. and EATON, J.C. (1938) : Quart. J. Med. 7, 271.

GOVAERTS, P. and LAMBERT, P-P. (1949) : Acta clin. belg. 4, 341.

GURIN, S., BACHMAN, C. and WILSON, D.W. (1940) : J. Biol. Chem., 133, 467.

HAMILTON, W.J., BOYD, J.D. and MOSSMAN, H.W. (1945) : Human Embryology, Cambridge, Heffer, p. 26.

HANSEN, P.G., JACOBSEN, E.A. and PETERSEN, M.F. (1943) : Acta physiol. scaninav., 6, 195.

HARRISON, G.A. (1947) : Chemical Methods in Clinical Medicine, 3rd. Ed., London, Churchill, p. 121.

HANK, P.B., OSER, B.L. and SUMMERSON, W.H. (1947) : Practical Physiological Chemistry, 12th Ed., London, Churchill, p. 779.

HOET, J.P. (1954) : Diabetes 3, 1.

- - (1957) : W.H.O. Symposium on the Public Health Aspects of Chronic Disease, Amsterdam.

HURWITZ, D. and JENSEN, D. (1946) : New England J. Med. 234, 327.

INGLE, D.J. (1941a) : Endocrinology 29, 649.

- - (1941b) : Am. J. Med. Sci. 201, 153.

- - , Li, C.H. and EVANS, H.M. (1946) : Endocrinology 39, 32.

JABLOKOW, V., HUTCHINS, M. and KNIGHTS, E.M. (1957) : Diabetes 6, 426.

JACKSON, W.P.U. (1952) : Brit. Med. J., 2, 690.

JOHN, H.J., (1930) : M. J. and Rec. 131; 287, 351, 398.

JOHNSON, D.G. and BOSNES, R.W. (1948) : J. Clin. Invest. 27, 745.

JONES, M.S. and MACGREGOR, T.N. (1936) : Lancet, 2, 974.

JORGENSEN, S. (1927) : Acta. med. scand., 65, 116.

JOSLIN, E.P. (1959) : The Treatment of Diabetes Mellitus, 10th Ed., London, Kimpton, p. 11.

KAUFMANN, E. and KÜSTER, W. (1926) : Deutsche med. Wehnschr. 2, 1722 ; cited by Skipper, (1933).

KIMBALL, S. (1932) : Guy's Hosp. Rep. 82, 157.

KRISS, J.P. and FUTCHER, P.H. (1948) : J. Clin. Endocrinol. 8, 380.

LABBÉ, M. and CHEVKI, M. (1926) : Comp. rend. soc. biol. 94, 302.

LAMBIE, C.G. (1926) : J. Obstet. Gynaec. Brit. Emp. 33, 563.

LEWIS, T.L.T. (1956) : Progress in Clinical Obstetrics and Gynaecology, London, Churchill, p. 111.

LISLE, W.J. and DICK, G.F. (1932) : Arch. Int. Med. 50, 801.

LORAINE, J.A. (1949a) : J. Obstet. Gynaec. Brit. Emp. 56, 1051.

- - (1949b) : Brit. med. J. 2, 1496.

LOZNER, L.L., WINKLER, A.W., TAYLOR, P.H.L. and PETERS, J.P. (1941) : J. Clin. Invest., 20, 507.

MACLEAN, H. and de WESSELOV, O.L.V. (1920-1) : Quart. J. Med., 14, 103.

MANN, F.C. and MAGATH, T.B. (1922) : Arch. Int. Med. 30, 73.

MARAZZINI, F. (1957) : Ann. Obstet. Ginec., 79, 63.

MAZER, C., MERANZE, D.R. and ISRAEL, S.L. (1935) : J. Amer. Med. Assoc., 105, 257.

MILLER, H.C., HURWITZ, D. and KUDER, K. (1944) : J. Amer. Med. Assoc., 124, 271.

MORARD, J. Cl., RUTISHAUSER, E., and CHATILLON, J. (1956) : J. urol. med. et chir. 61, 612.

MORRIS, C.J.O.R. (1955) : Brit. med. Bull., 11, 101.

MURDOCH, R. (1953) : J. Obstet. Gynaec. Brit. Emp. 60, 785.

NELSON, N. (1944) : J. Biol. Chem. 153, 375.

NELSON, W.O. and OVERHOLSER, M.D. (1936) : Endocrinology, 20, 473.

OAKLEY, W. (1953) : Brit. med. J. 1, 1413.

PARVIAINEN, S., SOIVA, K. and VARTIANEN, S. (1950) : Acta obstet. gynaec. scand. 29, supplement 5.

PEDERSEN, J. and BRANDSTRUP, E. (1956) : Lancet 1, 607.

PEEL, J.H. and OAKLEY, W. (1949) : Trans. 12th. Brit. Congr. Obs. Gyn.

PEEL, J.H. (1955) : Brit. med. J. 2, 870.

- (1959) : British Obstet. and Gynaecological Practice (Obstetrics) Edit. Holland, 2nd. Ed., London, Heinemann, p. 517.

RATHERY, F. and RUDOLPH, M. (1928) : Bull. et mém. Soc. méd. hôp de Paris, 52, 741.

ROLLAND, C. (1954) : Edin. med. J. 61, 257.

ROSENBERG, M. (1924) : Klin. Wehnschr. 3, 1561, cited by Skipper (1933).

ROSS, C.W. (1938) : Arch. Dis. Childh., 13, 289.

SANTOS, R.F., McCANCE, R.A. and RANDLE, P.J. (1955) : Nature, 176, 115.

SCHULLER, E. (1957) : Acta. Endocrin. 25, 345.

SCOTT, J.S. (1958) : J. Obst. Gynaec. Brit. Emp. 65, 689.

SELYE, H. (1947) : Textbook of Endocrinology, University of Montreal, p. 495 et seq.

SIMS, E.A.H. and KRANTZ, K.E. (1958) : J. Clin. Invest. 37, 1764.

SKIPPER, E. (1933) : Quart. J. Med. 2, 353.

SMITH, Homer W. (1951) : The Kidney - Structure and Function in Health and Disease, New York, Oxford University Press, p. 82 et seq.

SMITH, O.W. and SMITH, G van S. (1937) : Amer. J. Obstet. Gynaec. 33, 365.

- - - (1940) : Amer. J. Obstet. Gynaec. 39,405.

SOSKIN, S. and LEVINE, R. (1955) : Modern Nutrition in Health and Disease, Edit. Wohl and Goodhart, Philadelphia, Lea and Febiger, p. 160.

SPIEGELMAN, A.R. (1940) : Am. J. Med. Sci. 200, 228.

STEWART, C.F. SCARBOROUGH, H. and DAVIDSON, J.N. (1938) : Quart. J. Med. 7, 229.

STOWERS, J.M. (1961) : Brit. J. Clin. Pract. 15, 65.

THORPE, W.V. (1947) : Biochemistry for Medical Students, 4th Ed. London, Churchill, p.217.

TUCHMAN, H. (1937) : Compt. rend. soc. biol. 125, 635.

TUNBRIDGE, R.E. and ALLIBONE, E.C. (1949) : Quart. J. Med., 9, 11.

VARLEY, H. (1954) : Practical Clinical Biochemistry, London, Heinemann, p. 57.

VENNING, E.H. (1946) : Endocrinology, 39, 203.

WELSH, G.W. and SIMS, E.A.H. (1960) : Diabetes, 9, 363.

WHITE, P. (1947) : Penn. M.J. 50, 705.

(1956) : The Treatment of Diabetes Mellitus, 10th Ed., Edit. Joslin, London, Kimpton, p.690.

WILKERSON, H.L.C. and REMEIN, Q.R. (1957) : Diabetes 6, 324.

WILLIAMS, J. (1962) : Personal communication.

APPENDIX A.

The method of paper chromatography used for the identification of urinary sugars throughout this investigation, is that described by Williams (1954). The following description consists largely of extracts from the original article and the debt to the author is fully acknowledged:-

One invaluable application of chromatographic procedures is the identification of reducing sugar in urine. Most techniques, although simple, have the common disadvantage that solvents must be run for a long period in order to completely separate the carbohydrates which are most likely to be present, i.e. lactose, galactose, glucose, fructose, pentose. The following are details of an ascending technique adopted in this laboratory, (Rainhill Hospital, Near Liverpool), to reduce this time factor and yet to obtain complete separation of the suspected sugars. It is of interest because it:-

(a) Utilizes triangular shaped filter paper.

(b) Affords complete separation of reducing sugars within the comparatively short time of 10 hours.

(c) Requires a solvent front run of only 18 cm.

Equipment and Reagents Required.

Pipettes - Small pipettes calibrated to deliver 5 and 10 µl.

Apparatus - A duralumin frame which can support one dozen 20 x 20 cm. filter paper squares. The frame consists of two duralumin plates 20 x 20 cm. square, joined by four rods, 30 cm. long, which pass through holes drilled into the corners of each plate ; the latter are locked on to the rods with small nuts. Thirteen 2 cm. long duralumin rings are placed along each of the two upper rods in order to separate filter papers when the frame is in use.

A suitably sized, enclosed cabinet or glass aquarium tank is required to house the duralumin frame.

Filter Paper - Whatman No. 1 has been found quite suitable. A triangle is cut from a 20 x 20 cm. square as in Fig. 9.

Solvent - This is prepared by mixing three volumes of 1-butanol with two volumes of pyridine and 1.5 volumes of distilled water.

Developer - Benzidene reagent - a knifepoint of benzidene is dissolved in 2 to 3 ml. glacial acetic acid and volume made up to 20 ml. with absolute alcohol.

Control Solution:-

Lactose	1 gramme.
Galactose	1 "
Glucose	1 "
Laevulose	1 "
Xylose	1 "

Saturated benzoic acid 100 ml.

Method.

Urine is diluted with distilled water to approximately 1 per cent. concentration of reducing sugar. If sugar is less concentrated than 1 per cent. then a greater volume of urine is applied to the filter paper.

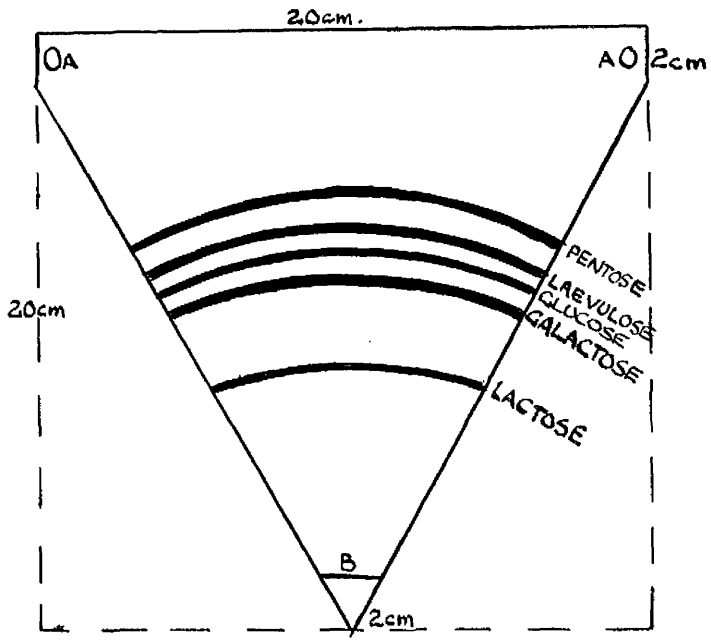


FIG. 9.

Five µl. amounts of urine and control solutions are applied on to separate papers at line B. The fluids must be applied in a continuous line and must touch both edges of the paper. Filter papers are allowed to dry at room temperature. The filter papers are supported on frame by passing upper bars through holes A.A., papers being separated by duralumin rings. The frame is placed in the cabinet and tips of the filter papers are dipped into solvent mixture contained in petri dish lid ; the solvent moves slowly up the filter paper, reaching the top edge within approximately 10 hours. For the sake of convenience papers may be left in the solvent from late afternoon until early next morning, when

they are removed and dried in a warm current of air.

To develop the spots the papers are thoroughly wetted by rapid immersion in benzidene-alcohol mixture and dried in a warm current of air. After drying they are placed in a hot air oven at 100°C. for 10 minutes when reducing sugars appears as brown arcs spreading from edge to edge of paper.

Urine sugar is identified by comparing its position with that of control sugars.

Shortly after this method was first adopted in the Research Laboratory of the Glasgow Royal Maternity Hospital, a slight modification was introduced. This consisted of mixing the urine with half its volume of Permutit "Bio-Deminrolit" mixed bed resin. The mixture was then centrifuged for the purposes of de-salting and to a lesser extent, for the removal of pigment. However this process made no appreciable difference to the resultant chromatograms and the modification was considered quite unnecessary and consequently discontinued.

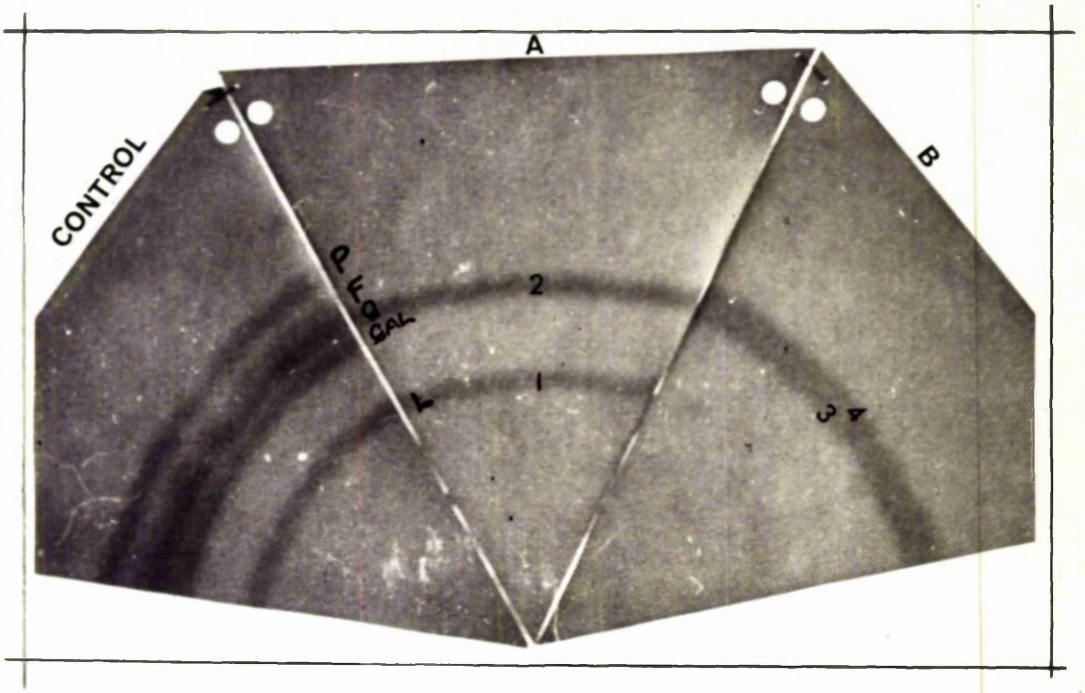


FIG. 10.

COMPLETED CONTROL AND TEST CHROMATOGRAMS A. & B.
(described opposite).

Results.

Fig. 9 shows the completed chromatogram prepared with the control solution. The reducing sugars are completely separated and appear on the chromatogram, from the point of origin B, in the following order : Lactose, galactose, glucose, laevulose and pentose.

Fig.10 shows 3 chromatograms which have been prepared in the manner described above. By applying Test Sheets A and B individually to the Control, the following sugars are identified:-

A. 1. Lactose.
2. Glucose.

B. 3. Glucose.
4. Fructose.

Reference.

Williams, R. (1954) : J. Med. Lab. Tech. 12, 43.

APPENDIX B.

Explanatory Note to Table A.

The results of the individual tests for urinary sugar described in the Preliminary Investigation (Section I) are reported serially under the corresponding week of gestation:

Negative results are shown 0

False positive results F.P.

The results obtained using either "Clinitest" (C) or Benedict's qualitative solution (B) are shown:- Negative - ; slight trace, sl.tr. ; trace, tr., and + , ++..., signifying sugar concentrations of approximately 50, 75 etc., mg. per 100 ml.

The results from Chromatography (Ch) are shown according to the sugar(s) present :

Glucose (G), Lactose (L), Galactose (Gal.), Fructose (F) and Pentose (P).

Where there are two or more sugars present concurrently and one of them is in appreciably higher concentration, this is signified by a + sign eg. $Ch\begin{pmatrix} G+ \\ L \end{pmatrix}$.

Table A (cont.) OUTPATIENT SERIES Multigravidae

Case No	Age	Week of Gestation 10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
19	24			O				O								F.P.																
20	26			F.P.																												
21	30			F.P.				O																								
22	30							O							O																	
23	23	O						O				F.P.				O				O				F.P.		O		O				
24	26			O				O							O							O									
25	22										O				O																	
26	30																															
27	25																															
28	21										O				F.P.				O							O						
29	33											O					O				O				O		O	O	O	O	O	O
30	23		O				O								F.P.																	
31	35						O				O										O			F.P.								
32	30	O			O				O								F.P.							O		O			O			
33	36																															
34	29						O									O			O													F.P.

TABLE A OUTPATIENT SERIES Primigravidae

Case No	Age	week of gestation 10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	17								○							○			·	○						F.P.	C Tr. B Tr. Ch G					C B Tr Ch {G L
2	25					○				○				C Tr. B Tr. Ch {G L		C − B Tr. Ch L							○		F.P.		○		○		
3	19										○			F.P.				F.P.							F.P.		○	○	C − B Tr. Ch. Gal.	○		
4	20				○				○				C − B Tr. Ch L					F.P.	C Tr. B Tr. Ch {G L			C − B Tr. Ch L			C Tr. B + Ch {G L				C − B Sl.Tr. Ch L			
5	21											○		○								C − B Tr. Ch L	C − B Tr. Ch L	C Tr. B Tr. Ch {G Gal.			F.P.		○		○	
6	21							F.P.				C − B Tr. Ch P					○				○			C − B Tr. Ch L		F.P.	C − B Sl.Tr. Ch Gal.		F.P.	F.P.	C − B Sl.Tr. Ch L	C − B Tr. Ch L
7	21									C − B Tr. Ch L						C − B Tr. Ch L				C + B ++ Ch {G+ L			C Tr. B Tr. Ch {G L		C − B Tr. Ch {G L		C − B Tr. Ch {G L	C Tr. B Tr. Ch {G L		C − B Tr. Ch L		C − B Tr. Ch {G L
8	40											C − B Tr. Ch G			○			C + B + Ch G			F.P.		C Tr. B Tr. Ch L		C − B Tr. Ch G	C − B Tr. Ch L	C − B Tr. Ch L	C − B Tr. Ch L	○	C − B Sl.Tr. Ch L		
9	32											○						F.P.			C − B Tr. Ch L		C Tr. B + Ch {G+ L		C Tr. B + Ch {G L		C − B Tr. Ch L	C − B Tr. Ch L	C − B Tr. Ch {G L	C − B Tr. Ch {G L	C Tr. B + Ch {G Gal.	C − B Sl.Tr. Ch L
10	28								C − B Tr. Ch G				C − B + Ch G				C − B Tr. Ch {G L				C − B Tr. + Ch G			C Tr. B + Ch {G L			C Tr. B + Ch {G L	C Tr. B Tr. Ch {G L		C Tr. B + Ch {G L
11	27	○			○			○			○				○				○				○		○		○			○	C − B Tr. Ch {G L	○	○
12	21									○					○				F.P.				C − B Sl.Tr. Ch {G Gal.			C − B Sl.Tr. Ch {G Gal.			F.P.		C − B Sl.Tr. B Tr. Ch L		
13	19			○			○			F.P.						○		F.P.			C − B Tr. Ch {G L			C Tr. B + Ch {G+ L	○			C − B Sl.Tr. Ch L				
14	28								C + B ++ Ch {G L									C Tr. B + Ch G				C + B ++ Ch G		C Tr. B + Ch {G+ L	C − B Sl.Tr. Ch {G L		C Tr. B + Ch {G+ L			C − B Sl.Tr. Ch {G L		
15	21							C Tr. B Tr. Ch {G L												○		F.P.	○						C − B Sl.Tr. Ch L		F.P.		
16	25											F.P.				○					○			C − B Tr. Ch L		○		○	○			
17	22		○													○				C − B Sl.Tr. Ch G			F.P.				C − B Tr. Ch G		C − B Sl.Tr. Ch G	C − B Tr. Ch G		C − B Tr. Ch G
18	22										○			○				○				F.P.		○			○	C − B Tr. Ch L	C B Tr + Ch L	C − B Tr. Ch L		